ORIGINAL ARTICLE

# Doxorubicin inhibits muscle inflammation after eccentric exercise

Sheng-Chih Huang[1†], Jin-Fu Wu[1], Suchada Saovieng[1], Wei-Horng Chien[1], Ming-Fen Hsu[1], Xiao-Fei Li[1], Shin-Da Lee[2], Chih-Yang Huang[3], Chih-Yang Huang[4,5†] & Chia-Hua Kuo[1,2]*

[1]Laboratory of Exercise Biochemistry, University of Taipei, Taipei, Taiwan; [2]Graduate Institute of Physical Therapy and Rehabilitation Science, China Medical University, Taichung, Taiwan; [3]Translation Research Core, China Medical University Hospital, Taichung, Taiwan; [4]Department of Healthcare Administration, Asia University, Taichung, Taiwan; [5]Graduate Institute of Basic Medical Science, China Medical University, Taichung City, Taiwan

## Abstract

**Background**    Doxorubicin, a widely used anti-tumour drug, is known to cause muscle loss in cancer patients.
**Methods**    Following an acute dose of doxorubicin injection (2.5 mg/kg per body weight), we examined macrophage distribution in rat soleus muscle challenged by eccentric exercise (downhill running). Long-term doxorubicin treatment (one injection every 3 days) on muscle mass and survival were also determined.
**Results**    Under non-exercised condition, increased tumour necrosis factor (TNF)-alpha mRNA and decreased IL-10 mRNA were observed in soleus muscle of doxorubicin-treated rats, compared with saline-treated control rats. However, increases in inflammation score (leukocyte infiltration), nitrotyrosine level, and M1 macrophage (CD68^{+}) invasion in exercised soleus muscle were absent in doxorubicin-treated rats, whereas increased M2 macrophage (CD163^{+}) localization in exercised muscle was less affected by doxorubicin. Despite coenzyme Q (Q10) supplementation significantly elevated TNF-alpha mRNA, nitrotyrosine, and anti-oxidant gamma-glutamylcysteine synthetase (GCS) levels in non-exercised soleus muscle, these pro-inflammatory responses were also abolished in doxorubicin-treated rats. Results from long-term doxorubicin treatment show a significant muscle loss followed by an accelerated death, which cannot be reversed by Q10 supplementation.
**Conclusions**    (i) Doxorubicin impairs inflammation mechanism by depleting M1 macrophage in exercised skeletal muscle; (ii) Muscle loss and accelerated death during prolonged doxorubicin treatment cannot be reversed by Q10 supplementation.

**Keywords**    Muscle atrophy; Chemotherapy; Adriamycin; Eccentric exercise; Skeletal muscle; Macrophage; CD68; CD163

## Introduction

Doxorubicin has been used extensively for chemotherapy of cancer by the mechanism through which it systemically inhibits cell proliferation *in vivo*. One of the most devastating symptoms associated with mortality of cancer patients during the chemotherapy is development of sarcopenia.[1] In humans, survival time in patients who were developed sarcopenia was significantly shorter than in patients who were not developed sarcopenia.[2] Doxorubicin has been reported to cause muscle weakness and increase pro-inflammatory tumour necrosis factor-alpha (TNF-alpha) level.[3] Evidence from tumour-free animal study suggests that the sarcopenic effect of doxorubicin is not associated with tumour growth.[1]

Muscle size is maintained by a dynamic balance between muscle loss and regeneration. A restoration mechanism to encounter increased entropy of recruited muscle during daily physical challenge is essential, which is currently found to associate with macrophage-mediated inflammation.[4–6] Macrophage functions to recognize unhealthy cells, activate phagocytosis, regenerate tissue, and finally resolve inflammation.[4,7] Distribution of M1 (CD68^{+}) and M2 (CD163^{+}) macrophages in inflamed muscle tissue regulates the development of skeletal muscle after damage.[8,9] M1 macrophage

is responsible for phagocytic events during early phase of inflammation, and M2 macrophage presents during regenerative phase of inflammation.[4]

Q10 is currently considered as an adjuvant option in hope to minimize unwanted side effect during doxorubicin-based anticancer therapy,[10] according to previous results on decreased toxicity of multiple organs during doxorubicin treatment.[11,12] How doxorubicin may influence macrophage distribution during recovery process of eccentric exercise-challenged skeletal muscle and whether Q10 supplementation can modulate this influence have not yet been examined. Exercise containing eccentric muscle contraction (such as downhill running and resistance exercise) has been shown to cause muscle inflammation,[13] which is also known to be an effective way to increase muscle mass during training.[14] In this study, we hypothesized that (i) doxorubicin can alter distribution of M1 macrophage and M2 macrophage in skeletal muscle after eccentric exercise; (ii) Q10 supplementation can modulate inflammation, prevent muscle loss, and increase survival rate in doxorubicin-treated tumour-free rats.

# Methods

## Animals

This study was conducted in accordance to guidelines in the care and use of animals conformed to Taiwan government law, and was approved by the Animal Care and Use Committee at University of Taipei (approval number 102001). All Sprague Dawley rats (aged between 4 and 5 months) were obtained from LASCO Corporation (Taipei, Taiwan). Sixty four male Sprague Dawley (SD) rats (body weight $534 \pm 5$ g) were used for exercise challenge study to trigger muscle inflammation. Fifty six rats (body weight $485 \pm 4$ g) were used for survival experiment, to determine the protective effect of Q10 on survival during a long-term doxorubicin treatment. All rats from above studies were evenly allocated into the following four groups: Control (Con), Doxorubicin (Dox), Q10, and Dox + Q10. Two animals were housed per cage with standard laboratory chow (PMI Nutrition International, Brentwood, MO, USA) and tap water *ad libitum*. All animals were kept in an animal room with a 12/12 h light/dark cycle, $23 \pm 2$ °C and 50% relative humidity for approximately one month until experiment.

## Doxorubicin and Q10

To determine the effect of doxorubicin and Q10 on eccentric exercise-induced muscle inflammation, a dose of doxorubicin (Actavis, Italy SpA., Milano, Italy) at 2.5 mg per kg body weight was intraperitoneal injected according to a method reported elsewhere,[15] and a dose of Q10 (PharmaEssentia,

Taipei, Taiwan) at 10 mg per kg body weight was orally delivered by stomach tube in solution.[11]

## Exercise challenge

Half of rats from each group were challenged to a downhill treadmill running according to previously published methods (Yu *et al.*, 2014). One week prior to experiments, all animals were acclimated to running on a rat treadmill at 10 m/min for 10 min per day. Rats were subjected to an acute bout of intermittent running at a 16° decline. Exercises consisted of 18 sessions (5 min/session) separated by 2 min of rest at 16 m/min for a total of 90 min. An acute dose of doxorubicin or Q10 was delivered 16 h before exercise challenge. Twenty four hours after exercise, rats were anaesthetized with intraperitoneal injection of Zoletil (50 mg per kg body weight). Soleus muscles were surgically collected from both legs and frozen immediately with liquid nitrogen and then stored at −80 °C for further histology, immunohistochemistry (IHC), and western blotting analysis.

## Survival experiment

Rats were used to determine whether oral Q10 supplementation has beneficial effect for doxorubicin treated rats. Q10 was orally delivered at 0930 am daily, whereas doxorubicin was injected every 3 days. Kaplan–Meier curves were plotted at the end of experiment.

## Body composition

Muscle mass was measured using dual-energy X-ray absorptiometry (DXA) (Lunar iDXA, GE Medical Systems, Madison, WI), operated with software version 13.60.033 (encore 2011).

## Histology and immunohistochemistry (IHC)

Histology and IHC staining were carried out by a professional pathologist at China Medical University Hospital (Taichung, Taiwan). Soleus muscle was fixed in 4% buffered formalin and embedded in paraffin. Tissue sections were cut in 2 to 5 μm slices and transferred onto coated slides (Super Frost Plus, Braunschweig, Germany). Antigen retrieval occurred in boiled water for 15 min in 0.1 M sodium citrate (pH 7.2). These pretreated slides were blocked for 15 min at room temperature with 5% BSA and then incubated at 4 °C overnight with primary antibodies: antibodies against rat CD68 (dilution 1:50) (abcam, Cambridge, UK) and rabbit CD163 (dilution 1:50) (AbD Serotec, Kidlington, UK). Briefly, specific antibody was purchased to perform IHC staining by using horseradish peroxidase-conjugated

avidin biotin complex (ABC) from the Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) and DAB chromogen (Vector Laboratories).[13] The sections will be counterstained with haematoxylin and mounted. Inflammation grade was evaluated using a standard semiquantitative scoring system, score 0: none; score 1: giant cells, lymphocytes, plasma cells; score 2: giant cells, eosinophil, neutrophil; score 3: many inflammatory cells, microabscess.

## Western blotting analysis

Soleus muscle (~100 mg) was homogenized in 1 mL of HES buffer containing 20 mM Hepes (Sigma-Aldrich, St Louis, MO, USA), 1 mM EDTA (Sigma-Aldrich, St Louis, MO, USA), and 250 mM sucrose (Sigma-Aldrich, St Louis, MO, USA). Homogenates containing 50 μg of protein were fractionated by SDS-PAGE (10–12% acrylamide) and transferred to PVDF membranes (PALL Life Science, Ann Arbor, MI, USA) by standard wet transfer methods. After 1 h blocking in PBS containing 5% skim milk, membranes were incubated with primary antibody overnight at 4 °C. Primary antibodies against nitrotyrosine were purchased from Millipore (Bedford, MA, USA) (dilution 1:1000); antibodies against manganese-dependent superoxide dismutase (MnSOD) (dilution 1:5000) were purchased from Enzo Life Sciences (Ann Arbor, MI, USA); and antibodies against GCS (dilution 1:100) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody-bound protein was detected using a peroxidase-conjugated anti-mouse secondary antibody (Sigma, St Louis, MO, USA) or anti-rabbit IgG (Cell Signaling Technology, Beverly, MA, USA) and enhanced chemiluminescent HRP substrate (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA). To verify equal protein loading, GAPDH (Sigma, St Louis, MO, USA) (dilution 1:5000) was used as an internal control. Western blot bands were quantified using a ChemiDoc™ XRS+ System (BioRad, Hercules, CA, USA).

## Quantitative polymerase chain reaction (qPCR)

Total RNA was isolated using Qiagen RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia, CA, USA). Reverse transcription of RNA was carried out using iScript™ cDNA Synthesis Kit (BioRad, Hercules, CA, USA). The following primer and probe sequences were used for amplification of target genes: TNF-α (NM_012675) forward primer: GAGTCATTGCTCTGTGAG; reverse primer: CTCTGAGGAGTAGACGATA; probe: CTGGCG TGTTCATCCGTTCTCT. IL-10 (L02926) forward primer: GATCCAGAGATCTTAGCTA; reverse primer: CTGAGGTAT CAGAGGTAA; probe: AACCTCGTTTGTACCTCTCTCCAA. TaqMan probes contained a FAM reporter at the 5′-end and a TAMRA quencher at the 3′-end. The RT-qPCR conditions have been previously described.[16] Gene relative expression levels

were determined by the change in crossing points of reaction amplification (ΔCt value) between targets mRNA and 18S mRNA for each treatment compared with the control group.

## Statistical analysis

Analysis of variance (ANOVA) was used to compare group difference for all variables. A two-way ANOVA was used to determine the main effect and interactive effect of treatments. A one-way ANOVA with a repeated measure was used to compare the mean differences in muscle mass between pre and post during long-term doxorubicin treatment. Fisher's protected least significant test, which holds the value of type I error to 0.05 for each test, was used to distinguish the differences between pairs of groups. A level of $P < 0.05$ was considered significant on all tests, and all values are expressed as means ± standard errors.

**Figure 1** Survival and muscle mass. Q10 supplementation failed to prevent accelerated death (A) and muscle loss (B) of rats during doxorubicin treatment (one injection every 3 days). Doxorubicin treatment resulted in a significant muscle loss in only 14 days, and Q10 supplementation failed to reverse this loss. * Significant difference against Pre, $P < 0.05$; † significant difference against Con group, $P < 0.05$. Abbreviation: Con, Control ($N = 14$); Q10, Coenzyme Q10 ($N = 14$); Dox, Doxorubicin; Q10 + Dox ($N = 14$), Coenzyme Q10 + Doxorubicin ($N = 14$).
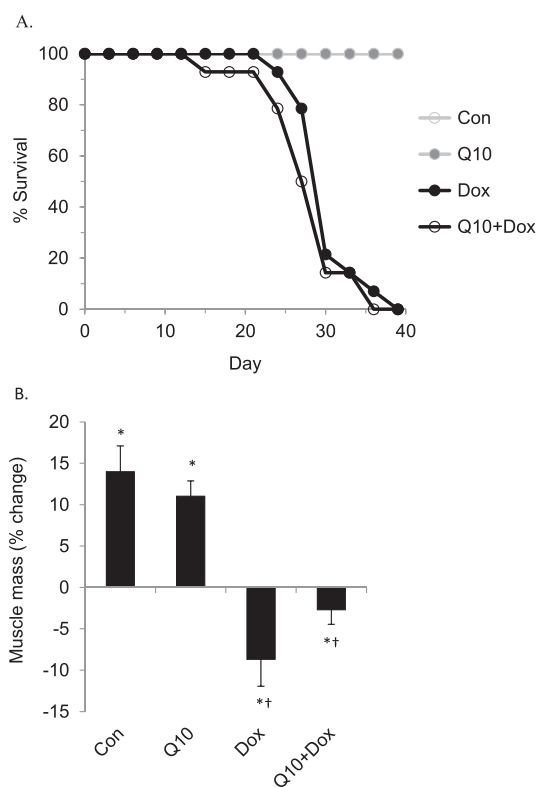
# Results

All rats treated with doxorubicin (at dose of 2.5 mg/kg per body weight every 3 days) died within 40 days (*Figure* 1A). No protective effect of Q10 supplementation (10 mg per kg body weight) was found during the entire treatment period. Muscle mass in doxorubicin-treated rats decreased significantly below those in saline-injected control rats at Day 14 of the treatment (*Figure* 1B).

An acute bout of downhill running resulted in increases in inflammation score (*Figure* 2A) and necrotic fibres

(*Figure* 2B) of soleus muscle, observed 24 h after the challenge. This increase was absent in doxorubicin-treated exercised rats. Centronucleation (a marker of muscle fibre regeneration) of soleus muscle was not changed in normal rats observed 24 h following eccentric exercise. However, a significant decreased centronucleation of exercised soleus muscle was observed in doxorubicin-treated rats (*Figure* 2C).

IHC results on CD68^{+} cell staining show a similar trend to inflammation score among four groups (*Figure* 3A). Exercise increased CD163^{+} cells, but this increase was not depleted

**Figure 2** Muscle inflammation. An acute injection of doxorubicin abolished inflammation of soleus muscle 24 h after eccentric exercise. Inflammation score was determined by pathologist based on haematoxylin and eosin (H&E) staining (A). Score 0: none; score 1: giant cells, lymphocytes, plasma cells; score 2: giant cells, eosinophil, neutrophil; score 3: many inflammatory cells, microabscess. An acute injection of doxorubicin inhibited necrosis (B) and lowered centronucleation (C) of soleus muscle 24 h after eccentric exercise. Representative histochemistry analysis of muscle sections from soleus muscle with H&E staining (Left side of bar chart). Arrowheads indicate leukocyte invasion (B) and centronucleation (C). Nucleolus is labelled with eosin staining (blue colour). Original magnification was 400×, scale bar 50 μm. * Significant difference against Con group, $P < 0.05$. Abbreviation: Con, Control; Q10, Coenzyme Q10; Dox, Doxorubicin; Q10 + Dox, Coenzyme Q10 + Doxorubicin.
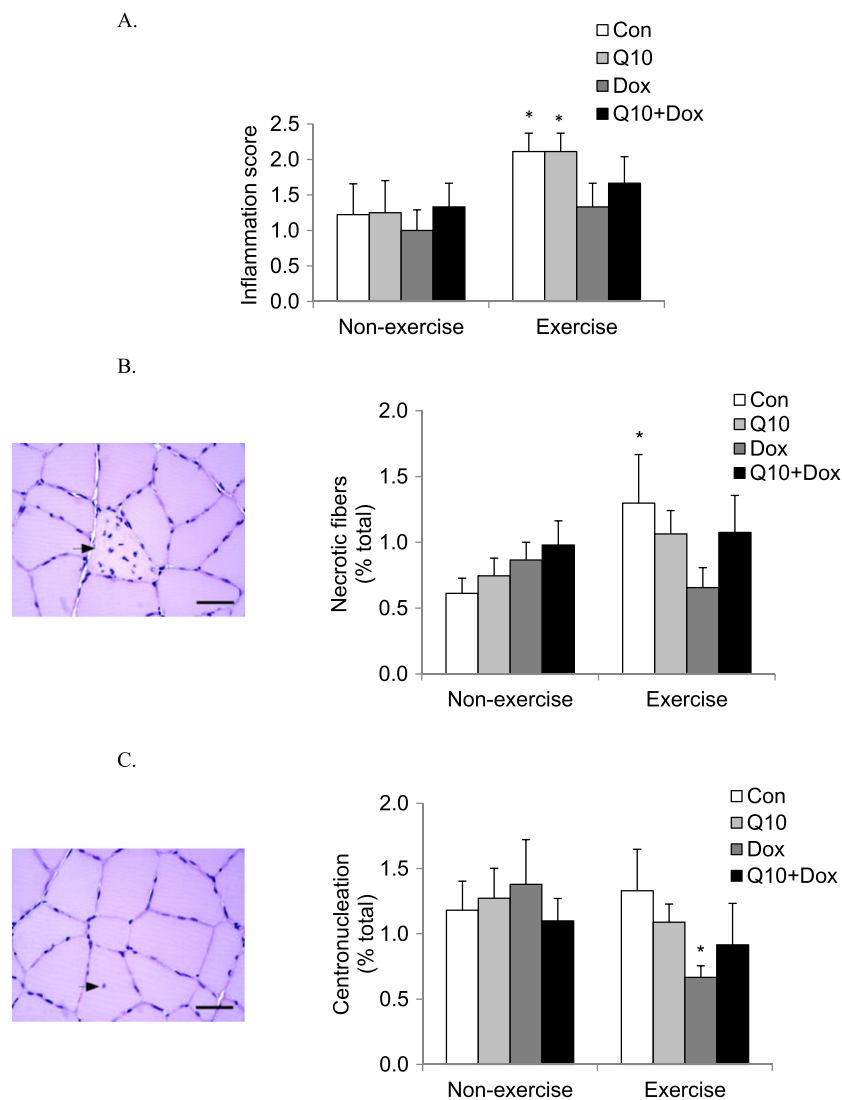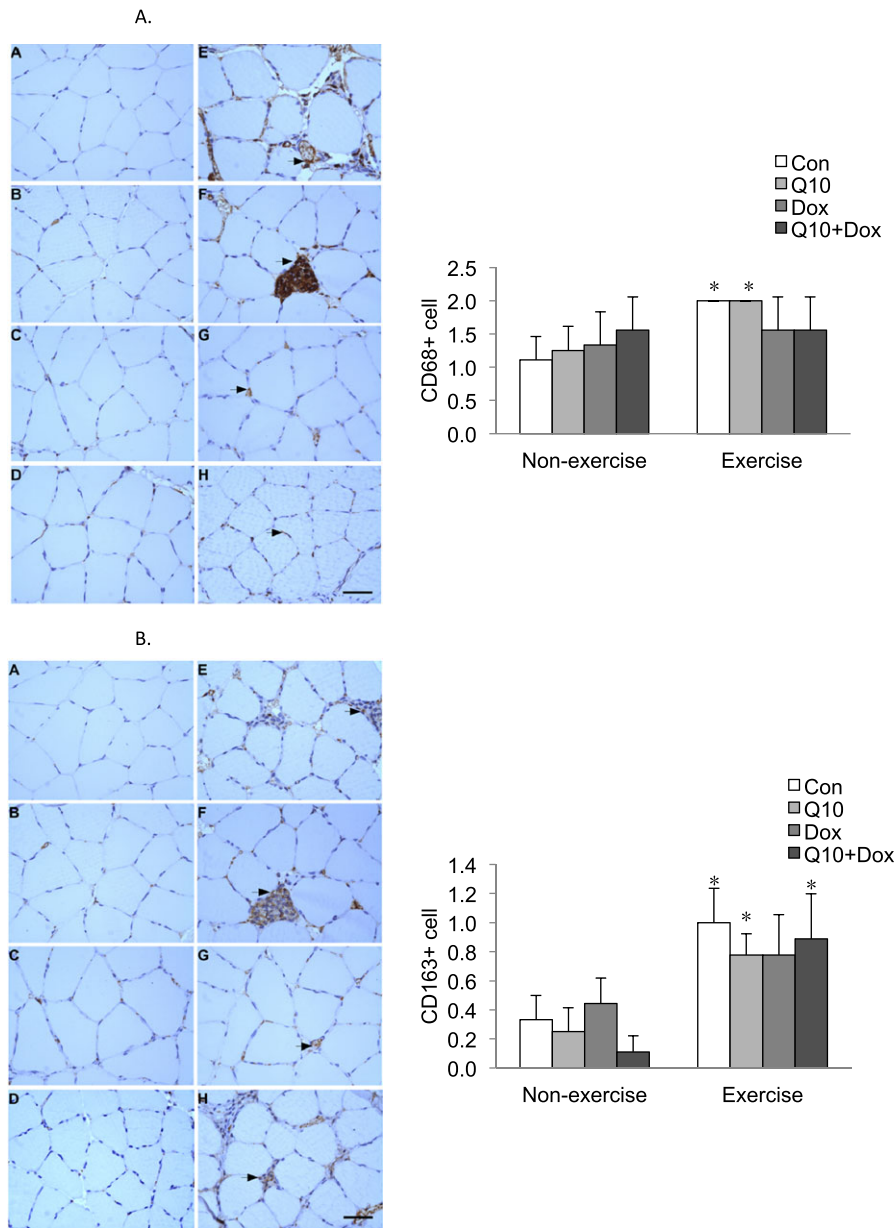
**Figure 3** Macrophage infiltration. Immunohistochemical staining shows a selective depletion of M1 macrophage (CD68^{+}) in acute doxorubicin treatment (A) but not M2 macrophage (CD163^{+}) (B). * Significant difference against Con group, $P < 0.05$. Abbreviation: Con, Control; Q10, Coenzyme Q10; Dox, Doxorubicin; Q10 + Dox, Coenzyme Q10 + Doxorubicin.



in doxorubicin rats (*Figure* 3B). TNF-alpha mRNAs in non-exercised soleus muscle of Q10, doxorubicin, and doxorubicin + Q10 groups were significantly increased above saline control group (*Figure* 4A), in the absence of increased leukocyte infiltration. No additive effect of doxorubicin and Q10 on mRNAs of both inflammatory mediators (TNF-alpha and IL-10) was found. Exercise increased TNF-alpha mRNA in muscle of both doxorubicin-treated and saline-control rats above non-exercised control level. Decreased IL-10 mRNA in exercised muscle below non-exercised control level occurred in both doxorubicin-treated and saline-treated rats (*Figure* 4B). Nitrotyrosine (*Figure* 5A) and GCS (*Figure* 5B) levels of soleus muscle were changed in similar to inflammation score among groups. In particular, exercise-induced increases in nitrotyrosine and GCS were absent in muscle among doxorubicin-treated rats. Antioxidant enzyme MnSOD was not different among four groups in both exercised and non-exercised muscles (*Figure* 5C).

**Figure 4** Tumour necrosis factor (TNF)-alpha and IL-10 mRNA. Eccentric exercise increased TNF-alpha mRNA and decreased IL-10 mRNA of soleus muscle. Q10 and doxorubicin had similar effect on TNF-alpha mRNA in non-exercised muscle. * Significant difference against Con group, $P < 0.05$; † significant difference against Q10 group, $P < 0.05$. Abbreviation: Con, Control; Q10, Coenzyme Q10; Dox, Doxorubicin; Q10 + Dox, Coenzyme Q10 + Doxorubicin.
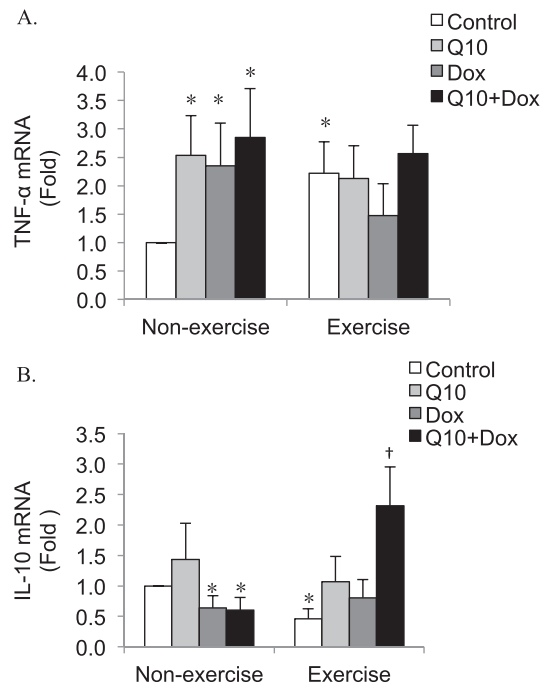
**Figure 5** Protein nitration, manganese-dependent superoxide dismutase (MnSOD), and gamma-glutamylcysteine synthetase (GCS). An acute injection of doxorubicin abolished increased in nitrotyrosine (A) and GCS (B) levels of soleus muscle induced by eccentric exercise, but MnSOD remained unaffected by both treatments (C). * significant difference against Con group, $P < 0.05$. Abbreviation: Con, Control; Q10, Coenzyme Q10; Dox, Doxorubicin; Q10 + Dox, Coenzyme Q10 + Doxorubicin.
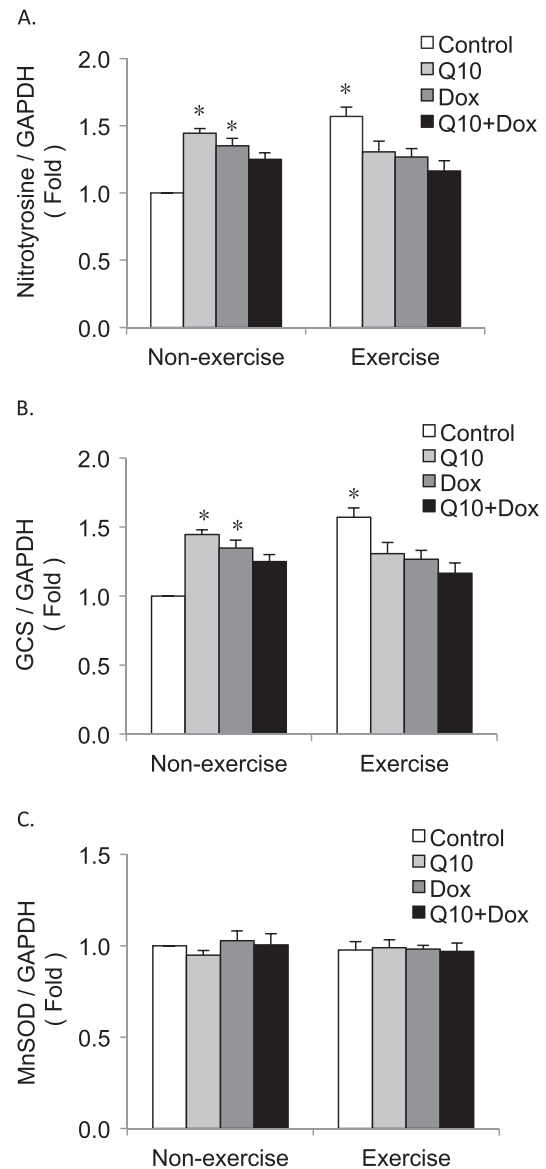


## Discussion

Inflammation is a wound-healing mechanism in living multicellular organisms.[4] During muscle inflammation, macrophages localize in damaged tissues.[9,17] Two subpopulations of macrophage take part during inflammation: (i) the early-invading, phagocytic macrophages (M1) reaches their highest concentration around 24 h following challenge and rapidly declines in 48 h[9]; (ii) the non-phagocytic macrophage (M2) that are distributed near regenerative fibres for several days. The major finding of this study is the inhibition of exercise-induced muscle inflammation by an acute dose of doxorubicin administration. IHC staining analysis confirms that M1 macrophage (CD68^{+}) was selectively depleted in exercised soleus muscle, 24 h after eccentric exercise challenge. Inability to clear unhealthy muscle fibres in exercised muscle because of M1 macrophage depletion may explain the observed lower ratio of regenerative muscle fibre with doxorubicin treatment. The suppressive effect of doxorubicin on inflammation is further supported by nitrotyrosine data.[18] Inhibiting nitric oxide production is expected to decrease stem cell recruitment to damaged tissue during regenerative phase of inflammation,[19] which may also contribute to the reduced ratio of regenerative fibres after eccentric exercise.

Doxorubicin is an inhibitor for cell proliferation, which has been widely used to inhibit proliferating tumour cells in cancer patients. Muscle mass is dynamically maintained by a balance of cell death and regeneration. Therefore, systemically inhibition of cell proliferation decreases muscle mass as doxorubicin treatment continues. In adults, cell turnover in muscle tissue is continuously occurring.[20] From our centronucleation results, approximately 1.33% of muscle fibre is renewing in eccentrically contracted soleus muscle. An acute dose of

doxorubicin injection decreased this percentage to 0.67%. However, the anti-proliferative action of doxorubicin is non-specific. Long-term systemic inhibition on cell proliferation will lead to tissue atrophy to most cell types in the body. Cells with high turnover rate, such as gastrointestinal epithelial cells, will particularly suffer and thus deteriorate nutrient absorption.[21] This may add to the devastating consequence of muscle loss during chronic doxorubicin treatment.

Another novel finding of the study is that exercise-induced increase in TNF-alpha mRNA and decrease in IL-10 mRNAs occur in the absence of increased leukocyte infiltration during acute doxorubicin treatment, suggesting that inhibiting leukocyte infiltration to exercised muscle sustains expression of inflammatory mediators in muscle tissue. It is possible that inhibiting cell regeneration by doxorubicin will cause population ageing of cells in muscle tissue. A substantial amount of muscle fibre is renewed during daily life of humans, evidenced by ^{14}C DNA measurement of skeletal muscle from two individuals aged 37 and 38 years old exhibiting an actual average muscle age of 15.1 years.[20] If cell regeneration mechanism is systemically blocked by doxorubicin, number of aged cell population will accumulate, which leads to increase TNF-alpha mRNA.[22] Doxorubicin has been shown to increase TNF-alpha mRNA levels of mice heart.[23] Inflammation is a process to trigger regeneration when cells produce danger-associated molecular pattern (likely the aged and unhealthy cells).[24] Because no significant increase in leukocyte infiltration in muscle tissue was found, the changes in TNF-alpha and IL-10 may simply mirror an imbalance between healthy vs. unhealthy (young vs. old) cell population resided in skeletal muscle caused by doxorubicin.

An unexpected new finding of the study is the increased baseline nitrotyrosine and TNF-alpha mRNA of skeletal muscle following Q10 supplementation, suggesting that Q10 is pro-inflammatory to skeletal muscle. However, existing literatures on Q10 supplementation show an attenuated protein nitration level[25] under increased oxidative stress induced by ischemic reperfusion and a suppressed TNF-alpha level in hyperglycemic heart.[26] Because none of those previous studies were conducted in normal (unchallenged) physiological condition, we speculate that Q10 may exert a hormesis effect by modulating inflammation to facilitate cell turnover (renewal). We have previously found that pre-treatment of pro-inflammatory components of ginseng eliminates exercise-induced muscle inflammation and protein nitration.[13] Furthermore, in this study, we hypothesized that Q10 supplementation can protect animals from lethal action of doxorubicin treatment, based on previous reports demonstrating its benefits on eliminating toxic action of doxorubicin on renal and testicular damage.[11] However, the results of current study fail to support this hypothesis.

The major limitation of this study is the lack of mechanistic insight into the role of mitochondria in the doxorubicin-induced muscle degeneration. Mitochondria dysfunction has been suggested as an underlying cause of doxorubicin-induced muscle degeneration and weakness.[27] Doxorubicin can intercalate into mitochondrial DNA[28] that may inhibit mitochondria biogenesis and results in accumulation of aged mitochondria.[29] Because mitochondrial turnover occurs at much faster pace compared with normal cells,[30] it is possible that increased reactive oxygen species because of accumulation of dysfunctional mitochondria is a cause of catabolic outcome of doxorubicin-induced muscle atrophy.[31,32]

Previous study in tumour-free model[1] has documented a significant muscle loss during doxorubicin treatment, which is apparently unrelated to tumour growth. Given the fact that muscle loss in both humans and animals are closely associated with increased mortality in both cancer patient[2] and elderly,[33,34] development of other adjuvant intervention to preserve muscle mass, other than Q10 supplementation, should be regarded as a primary goal of cancer patients under doxorubicin therapy.

## Conclusions

This is the first study demonstrating that doxorubicin blocks muscle inflammation induced by eccentric muscle contraction. Inflammation is crucial for muscle regeneration after physical challenge. Our results provide a new clue to implicate that imbalanced M1 and M2 macrophages in challenged muscle are involved with the development of muscle loss during anti-proliferation-based chemotherapy. Furthermore, although Q10 supplementation is pro-inflammatory to skeletal muscle, it cannot overcome muscle loss and accelerated death under long-term doxorubicin treatment.

## Acknowledgements

## Conflict of interest

Chia-Hua Kuo has received research grants from Pharma-Essentia, Taipei, Taiwan. Chih-Yang Huang has received

# References

1. Elsea CR, Kneiss JA, Wood LJ. Induction of IL-6 by cytotoxic chemotherapy is associated with loss of lean body and fat mass in tumor-free female mice. *Biol Res Nurs* 2015;**17**:549–557.

2. Tan BHL, Brammer K, Randhawa N, Welch NT, Parsons SL, James EJ, et al. Sarcopenia is associated with toxicity in patients undergoing neo-adjuvant chemotherapy for oesophago-gastric cancer. *Eur J Surg Oncol* 2015;**41**:333–338.

3. Gilliam LA, Moylan JS, Ferreira LF, Reid MB. TNF/TNFR1 signaling mediates doxorubicin-induced diaphragm weakness. *Am J Physiol* 2011;**300**:L225–L231.

4. Tidball JG. Inflammatory processes in muscle injury and repair. *Am J Physiol* 2005;**288**:R345–R353.

5. Tidball JG, Berchenko E, Frenette J. Macrophage invasion does not contribute to muscle membrane injury during inflammation. *J Leukoc Biol* 1999;**65**:492–498.

6. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. *Expert Rev Mol Med* 2011;**13**:e23.

7. Zhang X, Mosser DM. Macrophage activation by endogenous danger signals. *J Pathol* 2008;**214**:161–178.

8. Wang Y, Wehling-Henricks M, Samengo G, Tidball JG. Increases of M2a macrophages and fibrosis in aging muscle are influenced by bone marrow aging and negatively regulated by muscle-derived nitric oxide. *Aging Cell* 2015;**14**:678–688.

9. St Pierre BA, Tidball JG. Differential response of macrophage subpopulations to soleus muscle reloading after rat hindlimb suspension. *J Appl Physiol* 1994;**77**:290–297.

10. Swarnakar NK, Thanki K, Jain S. Enhanced antitumor efficacy and counterfeited cardiotoxicity of combinatorial oral therapy using Doxorubicin- and Coenzyme Q10-liquid crystalline nanoparticles in comparison with intravenous Adriamycin. *Nanomedicine* 2014;**10**:1231–1241.

11. El-Sheikh AAK, Morsy MA, Mahmoud MM, Rifaai RA, Abdelrahman AM. Effect of coenzyme-Q10 on doxorubicin-induced nephrotoxicity in rats. *Adv Pharmacol Sci* 2012;**2012**:8.

12. El-Sheikh AAK, Morsy MA, Mahmoud MM, Rifaai RA. Protective mechanisms of coenzyme-Q10 may involve up-regulation of testicular P-glycoprotein in doxorubicin-induced toxicity. *Environ Toxicol Pharmacol* 2014;**37**:772–781.

13. Yu SH, Huang CY, Lee SD, Hsu MF, Wang RY, Kao CL, et al. Decreased eccentric exercise-induced macrophage infiltration in skeletal muscle after supplementation with a class of ginseng-derived steroids. *PLoS One* 2014;**9**:e114649.

14. Roig M, O'Brien K, Kirk G, Murray R, McKinnon P, Shadgan B, et al. The effects of eccentric versus concentric resistance training on muscle strength and mass in healthy adults: a systematic review with meta-analysis. *Br J Sports Med* 2009;**43**:556–568.

15. Chicco AJ, Hydock DS, Schneider CM, Hayward R. Low-intensity exercise training during doxorubicin treatment protects against cardiotoxicity. *J Applied Physiol* 2006;**100**:519–527.

16. Chen CY, Tsai YL, Kao CL, Lee SD, Wu MC, Mallikarjuna K, et al. Effect of mild intermittent hypoxia on glucose tolerance, muscle morphology and AMPK-PGC-1alpha signaling. *Chin J Physiol* 2010;**53**:62–71.

17. Bréchot N, Gomez E, Bignon M, Khallou-Laschet J, Dussiot M, Cazes A, et al. Modulation of macrophage activation state protects tissue from ecnrosis during critical limb ischemia in thrombospondin-1-deficient mice. *PLoS One* 2008;**3**:e3950.

18. Gal A, Tamir S, Kennedy LJ, Tannenbaum SR, Wogan GN. Nitrotyrosine formation, apoptosis, and oxidative damage: Relationships to nitric oxide production in SJL Mice bearing the rcsX tumor. *Cancer Res* 1997;**57**:1823–1828.

19. K-i H, Ishibashi M, Ohtani K, Inoue S, Zhao Q, Kitamoto S, et al. Gene transfer of stromal cell–derived factor-1α enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. *Circulation* 2004;**109**:2454–2461.

20. Spalding KL, Bhardwaj RD, Buchholz BA, Druid H, Frisén J. Retrospective birth dating of cells in humans. *Cell* 2005;**122**:133–143.

21. Morelli D, Ménard S, Colnaghi MI, Balsari A. Oral administration of anti-doxorubicin monoclonal antibody prevents chemotherapy-induced gastrointestinal toxicity in mice. *Cancer Res* 1996;**56**:2082–2085.

22. Greiwe JS, Cheng B, Rubin DC, Yarasheski KE, Semenkovich CF. Resistance exercise decreases skeletal muscle tumor necrosis factor α in frail elderly humans. *FASEB J* 2001;**15**:475–482.

23. Pecoraro M, Del Pizzo M, Marzocco S, Sorrentino R, Ciccarelli M, Iaccarino G, et al. Inflammatory mediators in a short-time mouse model of doxorubicin-induced cardiotoxicity. *Toxicol Appl Pharmacol* 2016;**293**:44–52.

24. Kono H, Rock KL. How dying cells alert the immune system to danger. *Nat Rev Immunol* 2008;**8**:279–289.

25. Juan Y-S, Hydery T, Mannikarottu A, Kogan B, Schuler C, Leggett RE, et al. Coenzyme Q10 protect against ischemia/reperfusion induced biochemical and functional changes in rabbit urinary bladder. *Mol Cell Biochem* 2007;**311**:73–80.

26. Huynh K, Kiriazis H, Du X-J, Love JE, Gray SP, Jandeleit-Dahm KA, et al. Targeting the upregulation of reactive oxygen species subsequent to hyperglycemia prevents type 1 diabetic cardiomyopathy in mice. *Free Radic Biol Med* 2013;**60**:307–317.

27. Gilliam LA, Fisher-Wellman KH, Lin C-T, Maples JM, Cathey BL, Neufer PD. The anticancer agent doxorubicin disrupts mitochondrial energy metabolism and redox balance in skeletal muscle. *Free Radic Biol Med* 2013;**65**:988–996.

28. Ashley N, Poulton J. Mitochondrial DNA is a direct target of anti-cancer anthracycline drugs. *Biochem Biophys Res Commun* 2009;**378**:450–455.

29. Lebrecht D, Setzer B, Rohrbach R, Walker UA. Mitochondrial DNA and its respiratory chain products are defective in doxorubicin nephrosis. *Nephrol Dial Transplant* 2004;**19**:329–336.

30. Miwa S, Lawless C, Von Zglinicki T. Mitochondrial turnover in liver is fast in vivo and is accelerated by dietary restriction: application of a simple dynamic model. *Aging Cell* 2008;**7**:920–923.

31. Gilliam LA, Moylan JS, Patterson EW, Smith JD, Wilson AS, Rabbani Z, et al. Doxorubicin acts via mitochondrial ROS to stimulate catabolism in C2C12 myotubes. *Am J Physiol* 2012;**302**:C195–C202.

32. Gilliam LA, St. Clair DK. Chemotherapy-induced weakness and fatigue in skeletal muscle: the role of oxidative stress. *Antioxid Redox Signal* 2011;**15**:2543–2563.

33. Drescher C, Konishi M, Ebner N, Springer J. Loss of muscle mass: current developments in cachexia and sarcopenia focused on biomarkers and treatment. *J Cachexia Sarcopenia Muscle* 2015;**6**:303–311.

34. Kim JH, Lim S, Choi SH, Kim KM, Yoon JW, Kim KW, et al. Sarcopenia: an independent predictor of mortality in community-dwelling older Korean men. *J Gerontol* 2014;**69**:1244–1252.

35. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015. *J Cachexia Sarcopenia Muscle* 2015;**6**:315–316.