



Doxorubicin Induces Inflammatory Modulation and Metabolic Dysregulation in Diabetic Skeletal Muscle

Rashmi Supriya, Bjorn T. Tam, Xiao M. Pei, Christopher W. Lai, Lawrence W. Chan, Benjamin Y. Yung* and Parco M. Siu*

Department of Health Technology and Informatics, Faculty of Health and Social Sciences, The Hong Kong Polytechnic University, Hong Kong, China

OPEN ACCESS

Edited by:

Wataru Aoi,
Kyoto Prefectural University, Japan

Reviewed by:

Fan Ye,
University of Florida, USA
Feng He,
California State University, Chico, USA
Sampath K. Gollapudi,
Washington State University, USA

*Correspondence:

Benjamin Y. Yung
ben.yung@polyu.edu.hk
Parco M. Siu
parco.siu@polyu.edu.hk

Specialty section:

This article was submitted to
Striated Muscle Physiology,
a section of the journal
Frontiers in Physiology

Received: 18 May 2016

Accepted: 14 July 2016

Published: 27 July 2016

Citation:

Supriya R, Tam BT, Pei XM, Lai CW, Chan LW, Yung BY and Siu PM (2016) Doxorubicin Induces Inflammatory Modulation and Metabolic Dysregulation in Diabetic Skeletal Muscle. *Front. Physiol.* 7:323. doi: 10.3389/fphys.2016.00323

Anti-cancer agent doxorubicin (DOX) has been demonstrated to worsen insulin signaling, engender muscle atrophy, trigger pro-inflammation, and induce a shift to anaerobic glycolytic metabolism in skeletal muscle. The myotoxicity of DOX in diabetic skeletal muscle remains largely unclear. This study examined the effects of DOX on insulin signaling, muscle atrophy, pro-/anti-inflammatory microenvironment, and glycolysis metabolic regulation in skeletal muscle of db/db diabetic and db/+ non-diabetic mice. Non-diabetic db/+ mice and diabetic db/db mice were randomly assigned to the following groups: db/+CON, db/+DOX, db/dbCON, and db/dbDOX. Mice in db/+DOX and db/dbDOX groups were intraperitoneally injected with DOX at a dose of 15 mg per kg body weight whereas mice in db/+CON and db/dbCON groups were injected with the same volume of saline instead of DOX. Gastrocnemius was immediately harvested, weighed, washed with cold phosphate buffered saline, frozen in liquid nitrogen, and stored at -80°C for later analysis. The effects of DOX on diabetic muscle were neither seen in insulin signaling markers (Glut4, pIRS1Ser^{636/639}, and pAktSer⁴⁷³) nor muscle atrophy markers (muscle mass, MuRF1 and MAFbx). However, DOX exposure resulted in enhancement of pro-inflammatory favoring microenvironment (as indicated by TNF- α , HIF α and pNF κ Bp65) accompanied by diminution of anti-inflammatory favoring microenvironment (as indicated by IL15, PGC1 α and pAMPK β 1Ser108). Metabolism of diabetic muscle was shifted to anaerobic glycolysis after DOX exposure as demonstrated by our analyses of PDK4, LDH and pACCser⁷⁹. Our results demonstrated that there might be a link between inflammatory modulation and the dysregulation of aerobic glycolytic metabolism in DOX-injured diabetic skeletal muscle. These findings help to understand the pathogenesis of DOX-induced myotoxicity in diabetic muscle.

Keywords: type 2 diabetes mellitus, cancer chemotherapy, myotoxicity, anaerobic glycolysis, pro-inflammation, anti-inflammation

INTRODUCTION

Doxorubicin (DOX) is an effective chemotherapeutic drug for treating various types of cancer (Cortés-Funes and Coronado, 2007; Tacar et al., 2013). Nonetheless, the side effects of DOX on cardiomyocytes leading to life-threatening cardiomyopathy have limited its clinical use (Swain et al., 2003). Indeed, the adverse side effects of DOX have also been documented in the brain (Aluise et al., 2010), liver (Gokcimen et al., 2007), kidney (Wapstra et al., 1999), lung (Lim et al., 2010), blood vessels (Murata et al., 2001), and skeletal muscle (Smuder et al., 2011; Hayward et al., 2013). DOX is a potent drug for chemotherapy and it reflects a common picture of the chemotherapeutic drugs used for treating cancer (Shaikh and Shih, 2012). Generation of reactive oxygen species (ROS) and apoptosis-inducing characteristics are the common features shared by the anticancer drugs (Minotti et al., 2004; Nitiss, 2009; Gilliam and St. Clair, 2011). DOX has been demonstrated to adversely affect the muscle quality and muscle mass (Falkenberg et al., 2001; Gilliam and St. Clair, 2011; Smuder et al., 2011). DOX treatment was demonstrated to increase calcium flux from isolated sarcoplasmic reticulum vesicles in skeletal muscle cells (van Norren et al., 2009). DOX has also been shown to reduce calcium sensitivity by interfering with actin-myosin interaction in skeletal muscle (Hydock et al., 2011). Other adverse physiological consequences of DOX in skeletal muscle include muscle atrophy, muscle cell death, muscle mass reduction, and decrease in maximal twitch force (Falkenberg et al., 2001; Arthur et al., 2008; Gilliam and St. Clair, 2011; Smuder et al., 2011). DOX is comprised of a quinone moiety in its chemical structure, which is responsible for causing oxidative stress by interacting with molecular oxygen in skeletal muscle fibers (Chen et al., 2007). The resultant elevated oxidative stress disrupts several cellular mechanisms involving calpain and caspase-3 protease (Gilliam and St. Clair, 2011), AMP-activated protein kinase (AMPK) (Irrcher et al., 2008), and insulin receptor substrate 1 (IRS1) (Martins et al., 2012), in which DOX impairs the processes of apoptosis, autophagy, insulin signaling, and inflammatory pathways.

Inflammation and dysregulated glycolytic metabolism are reported in type 2 diabetes mellitus (Simoneau and Kelley, 1997; Pickup, 2004). Oxidative stress, inflammatory modulation and dysregulated glycolytic metabolism have also been reported in the DOX-exposed skeletal muscle (van Etten et al., 1998; Hardin et al., 2008; van Norren et al., 2009). Diabetes in skeletal muscle weakens the tricarboxylic acid (TCA) cycle (Ritov et al., 2010) and releases enormous amount of ROS by downregulating peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α) (Mootha et al., 2003; Sparks et al., 2005), which is one of the important mediators in the DOX-induced skeletal muscle myopathy (Min et al., 2015). ROS-PGC1 α signaling is a key pathway in the regulation of mitochondrial function in skeletal muscle and they are inversely related to each other (Liao, 2012). The augmented oxidative stress induced by DOX in diabetic skeletal muscle might cause metabolic dysregulation by inhibiting oxidative phosphorylation and upregulating lactate dehydrogenase (LDH) (Tannahill et al., 2013). The upregulated LDH leads to the

activation of hypoxia-induced factor 1 α (HIF1 α) (Tannahill et al., 2013) and nuclear factor kappa B (NF κ B) (Remels et al., 2014), which are transcription factors and regulatory molecules that favor toward pro-inflammatory microenvironment and trigger the secretion of pro-inflammatory cytokines [e.g., tumor necrosis factor alpha (TNF α) (Hotamisligil and Spiegelman, 1994) and Interleukin 6 (IL6) (Franckhauser et al., 2008)]. On the other hand, DOX has also been demonstrated to induce oxidative stress in skeletal muscle without altering LDH (van Norren et al., 2009) but to cause insulin resistance in muscle by downregulating IRS-1, glucose transporter type 4 (GLUT4), AMPK and glycogen synthase kinase 3 beta (GSK3 β) (Hayward et al., 2013). While AMPK downregulation is suggested to be involved in insulin resistance development in skeletal muscle treated with DOX (Viollet et al., 2009), AMPK also acts as a metabolic master switch that phosphorylates target proteins in fatty acid oxidation metabolic pathways in skeletal muscle (Viollet et al., 2009). Besides, AMPK acts as a regulatory molecule favoring the anti-inflammatory microenvironment leading to the shift toward oxidative metabolism (Sag et al., 2008), which reduces glycolytic rate by activating PGC1 α that ameliorates inflammatory cytokines/myokines such as TNF α and IL6 (Ostrowski et al., 1999; Steinberg et al., 2006) and enhances anti-inflammatory cytokines/myokines such as IL15 and IL10 (Wang et al., 2014; Crane et al., 2015).

Approximately 8–18% of patients with cancer are diabetic (Richardson and Pollack, 2005). Also, patients with diabetes are at a higher risk of developing cancers in breast, pancreatic, liver, kidney, endometrial, and colon (Richardson and Pollack, 2005). Though the exact links between diabetes and cancer are not known, the exposure to hyperglycemia, elevated insulin, and growth-promoting IGF-1 have been postulated to be the possible reasons explaining the increased incidence of cancers in diabetic patients (Grimberg, 2003). Nevertheless, patients with diabetes and cancer need to be particularly considered for undergoing chemotherapy due to the detrimental side effects of the chemotherapeutic drugs (Psarakis, 2006). In regard to DOX myotoxicity, DOX has been demonstrated to worsen insulin signaling, engender muscle atrophy, disseminate pro-inflammation, and induce an oxidative-to-glycolytic metabolic shift in normal skeletal muscle (Braun et al., 2014; Fabris and MacLean, 2015; de Lima Junior et al., 2016). However, the effects of DOX on skeletal muscle of diabetic individuals are largely unclear and the responsible molecular mechanisms in diabetic muscle remain to be elucidated. This study aimed to examine the effects of DOX on insulin signaling, muscle atrophy, pro-/anti-inflammatory microenvironment, and glycolysis metabolic regulation in skeletal muscle of diabetic animals.

METHODS

Animal

Male 14- to 18-week-old db/db mice were obtained from the Laboratory Animal Services Centre of The Chinese University of Hong Kong. The db/db mouse is a well-established leptin receptor-deficient animal model (homozygous allelic deficient

for the leptin receptor gene) that mimics the disease phenotype of human type 2 diabetes mellitus. Non-diabetic db/+ mice (heterozygous allele deficient for the leptin receptor gene) of the same genetic background as db/db mice were used as control group. Mice were housed in a humidity- and temperature-controlled environment and were exposed to a 12:12-h light:dark cycle in the Centralized Animal Facilities of The Hong Kong Polytechnic University. Mice were allowed to have access to standard animal diet and water ad libitum. Animal ethics approval was obtained from the Animal Ethics Sub-committee of The Hong Kong Polytechnic University.

Experimental Protocol

Non-diabetic db/+ mice and diabetic db/db mice were randomly assigned to the following groups: db/+CON, db/+DOX, db/dbCON, and db/dbDOX ($n = 5$ per group). The diabetic status of our examined db/db mice was confirmed by the measurements of fasting blood glucose level (db/db mice vs. db/+ mice: 27.1 ± 1.0 mmol/L vs. 7.8 ± 0.5 mmol/L) and HbA1c level (db/db mice vs. db/+ mice: $7.0 \pm 0.4\%$ vs. $5.8 \pm 0.1\%$). Mice in db/+DOX and db/dbDOX groups were intraperitoneally injected at one time point with DOX (Pharmacia and Upjohn SpA, Milan, Italy) at a dose of 15 mg per kg body weight whereas mice in db/+CON and db/dbCON groups were injected with the same volume of saline instead of DOX (Yu et al., 2014; Pei et al., 2015). Five days after the DOX administration, the gastrocnemius was immediately harvested, weighed, washed with cold phosphate buffered saline (PBS), frozen in liquid nitrogen, and stored at -80°C for later analysis (Yu et al., 2014).

Protein Fraction Preparation

Protein fractions were extracted from muscle homogenates as previously described (Yu et al., 2014; Pei et al., 2015) Forty mg of sample tissue were minced and homogenized in ice-cold lysis buffer (10 mmol/L NaCl, 1.5 mmol/L MgCl_2 , 20 mmol/L HEPES, pH 7.40, 20% glycerol, 0.1% Triton X-100, and 1 mM dithiothreitol). Homogenates were subject to centrifugation at $875 \times g$ for 5 min at 4°C . The supernatant was obtained and subject to further centrifugation at $3500 \times g$ for 5 min at 4°C in which these procedures were repeated thrice. Finally, the supernatant was collected as the cytoplasmic protein fraction. Then, protease inhibitor cocktail (P8340, Sigma-Aldrich) was added to the cytoplasmic protein fraction. Protein concentration was quantified in triplicates by Bradford assay (Coomassie Protein Assay, Pierce) with bovine serum albumin used as the standard.

Western Blot Analysis

The protein abundances of insulin signaling markers (IRS-1^{636/639}, Akt⁴⁷³, GLUT4), muscle atrophy markers (MuRF1 and MAFbx), markers for pro-inflammatory favoring microenvironment (TNF α , IL6, HIF1 α , pNfKbP65), markers for anti-inflammatory favoring microenvironment (IL10, IL15, pAMPK β 1¹⁰⁸, PGC1 α) and metabolic regulators (PDK4, pACC⁷⁹, LDH) were evaluated by Western blotting. Forty micrograms of protein were denatured at 95°C for 5 min in Laemmli buffer with 5% β -mercaptoethanol. The protein

samples were subject to gel electrophoresis on 10% SDS-PAGE gel. Resolved proteins were then transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore) by using the Bio-Rad Mini-Protein II system. The membrane was then blocked with 5% skimmed milk powder in PBS/0.1% Tween-20 (PBST) followed by incubation with respective primary antibodies overnight at 4°C . The primary anti-bodies used for probing various markers, their sources and dilutions used are listed in the **Table 1**. Membranes were incubated with appropriate secondary antibodies (i.e., anti-mouse, anti-rabbit or anti-goat IgG horseradish peroxidase-conjugated antibodies; Cell Signaling, 1:4000) after washing. The immunoreactivity was determined using the ECL chemiluminescence reaction kit (Perkin Elmer) and the images were captured by Chemi Doc (Bio-Rad camera, USA). GAPDH was used as the internal loading control. The arbitrary units of the blot signal are presented as net intensity \times band area, normalized to the signal of GAPDH or the respective total protein for phosphorylation status.

Lactate Dehydrogenase (LDH) Activity

Lactate dehydrogenase (LDH) activity was determined by a commercially available kit (ab102526, Lactate Dehydrogenase Activity Colorimetric Assay Kit, Abcam). The assay was performed according to the manufacturer's instruction. In brief, LDH converts pyruvate into lactate that reduces the developer to a colored product with absorbance at 450 nm measured by a microplate reader (Infinite F200, Tecan, Switzerland).

TABLE 1 | List of antibodies used.

Antibody	Dilution factor	Source
Anti-phospho-IRS1 (Ser636/639) rabbit polyclonal	1:1000	2388, Cell signaling technology
Anti-phospho-Akt (Ser473) rabbit polyclonal	1:1000	9271, Cell signaling technology
Anti-Akt rabbit polyclonal	1:1000	9272, Cell signaling technology
Anti GLUT4 rabbit polyclonal	1:500	07-1404, Millipore
Anti-MuRF1 rabbit polyclonal	1:500	32920, Santa Cruz
Anti-MAFbx rabbit polyclonal	1:500	33782, Santa Cruz
Anti-phospho-AMPK β 1(Ser108) rabbit polyclonal	1:1000	4181, Cell signaling technology
Anti PGC1 α rabbit polyclonal	1:500	13067, Santa Cruz
Anti-IL10 goat polyclonal	1:1000	365858, Santa Cruz
Anti-IL15 goat polyclonal	1:1000	1296, Santa Cruz
Anti-phospho-NFk β p65 rabbit monoclonal	1:1000	3033, Cell signaling technology
Anti-HIF1 α rabbit polyclonal	1:500	10790, Santa Cruz
Anti-TNF α goat polyclonal	1:1000	52746, Santa Cruz
Anti-IL6 goat polyclonal	1:1000	1265, Santa Cruz
Anti-PDK4 (Thr410/403) goat polyclonal	1:1000	14495, Santa Cruz
Anti-phospho-ACC (Ser79) rabbit polyclonal	1:1000	3661, Cell signaling technology
Anti-LDH rabbit polyclonal	1:500	33781, Santa Cruz

Data Analyses

Statistical analyses were performed by using the SPSS 21.0 software package (IBM, Chicago, IL, USA). Normality tests were performed to examine data distribution. All data were expressed as mean \pm standard error of the mean (SEM). Two-way ANOVA was used to examine the interaction and main effects of the two experimental factors (i.e., diabetes and DOX) and subsequent Tukey's HSD post-hoc test was used to examine the simple effect if a significant interaction effect was found. Statistical significance was set at $p < 0.05$. Of note, the adopted sample size in the present study resulted in a statistical power of 80% or above for the assessments including muscle mass, IRS1, MAFbx, IL6, IL10, IL15, AMPK, PGC1 α , HIF1 α , and LDH activity.

RESULTS

No Exacerbating Effect of DOX Exposure on Insulin Signaling in Diabetic Muscle

The abundance of insulin signaling proteins including phosphor-IRS1Ser^{636/639}, phosphor-AKTSer⁴⁷³ and GLUT4 were measured in the gastrocnemius muscle. No interaction effect of diabetes and DOX was observed in all the three markers. We observed the main effect of diabetes for phosphor-IRS-1 ($P = 0.0001$) and the abundance of protein was significantly increased by 145% ($P = 0.0001$) in the gastrocnemius muscle of db/dbCON mice relative to that of db/+CON mice (**Figure 1A**). There was a main effect of diabetes for AKT ($P = 0.042$), and the protein abundance of AKT was significantly reduced by 61% ($P = 0.041$) in the muscle of db/dbCON mice relative to db/+CON mice (**Figure 1B**). We did not observe interaction effect of diabetes with DOX or significant main effect of diabetes in GLUT4 (**Figure 1C**).

No Exacerbating Effect of DOX Exposure on Muscle Atrophy in Diabetic Muscle

The abundance of muscle atrophy markers including MuRF1, MAFbx and muscle mass were measured in the gastrocnemius muscle. No interaction effect of diabetes with DOX was found for MuRF1. There was a significant main effect of diabetes for MuRF1 ($P = 0.022$) and a significant 48.28% ($P = 0.022$) increase in protein abundance of MuRF1 in muscle of db/dbCON mice relative to that of db/+CON mice (**Figure 2A**). We did not observe any significant interaction effect for MAFbx. Also, no significant main effect of diabetes was found in MAFbx (**Figure 2B**). No significant interaction effect was observed in the muscle mass. However, significant main effect of diabetes was observed in muscle mass reduction ($P = 0.0001$) and muscle mass was significantly decreased by 58% ($P = 0.0001$) in the gastrocnemius muscle of db/dbCON mice relative to that of db/+CON mice (**Figure 2C**).

DOX Caused Anti-Inflammatory Microenvironment Diminution and Pro-Inflammatory Microenvironment Augmentation in Diabetic Muscle

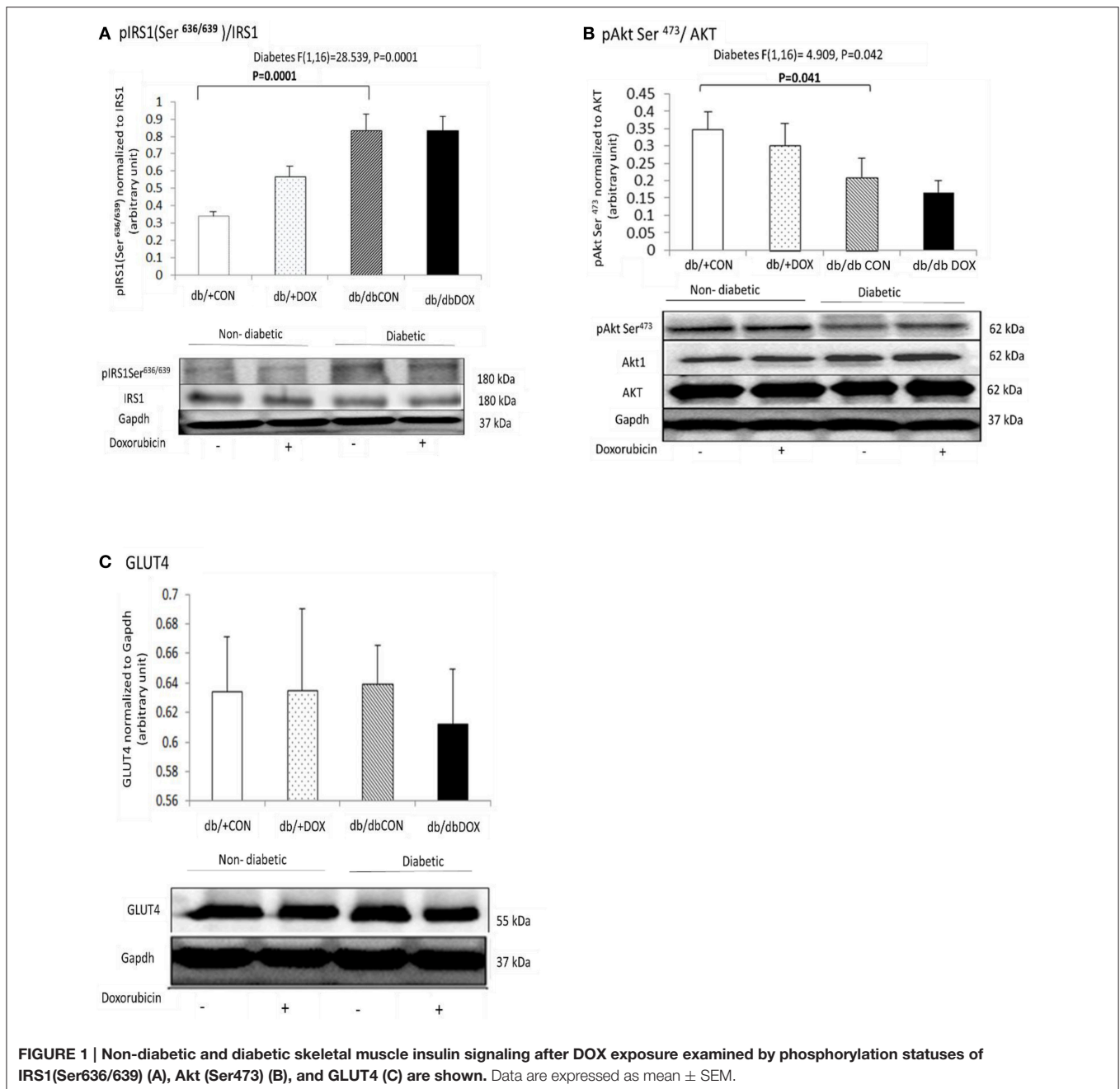
The muscle-specific cytokines, transcription factor and regulatory molecule that modulate anti-inflammatory

microenvironment were examined. No interaction effect of diabetes with DOX was observed for IL10. Significant main effect of diabetes was observed in IL10 ($P = 0.001$) and its protein abundance significantly decreased by 78% ($P = 0.001$) in the muscle of db/dbCON mice relative to that of db/+CON mice (**Figure 3A**). No interaction effect of diabetes with DOX was observed for IL15. Significant main effect of diabetes was found in IL15 ($P = 0.001$). The protein abundance of IL15 was significantly decreased by 51% ($P = 0.001$) in muscle of db/dbDOX mice relative to the muscle of db/+DOX mice and it was also significantly decreased by 66% ($P = 0.043$) in the muscle of db/dbDOX mice relative to db/dbCON mice (**Figure 3B**). Similarly, no interaction effect of diabetes and DOX was observed in AMPK. Significant main effect of diabetes was found in AMPK ($P = 0.0001$). There was a significant 62% ($P = 0.0001$) decrease in protein abundance of phosphor-AMPK β 1Ser108 in muscle of db/dbCON mice relative to db/+CON mice and was also significantly decreased by 57% ($P = 0.004$) in db/dbDOX mice relative to that of db/dbCON mice (**Figure 3C**). Significant interaction effect of diabetes with DOX ($P = 0.039$) for PGC-1 α was found. A significant 31% ($P = 0.001$) decrease in protein abundance of PGC-1 α in the muscle of db/dbCON relative to the muscle of db/+CON mice and also a significant 36% ($P = 0.031$) decrease in the muscle of db/dbDOX relative to that of db/dbCON mice was observed (**Figure 3D**).

The muscle-specific cytokines, transcription factor and regulatory molecule that modulate pro-inflammatory favoring microenvironment were assessed. No interaction effect of diabetes with DOX was observed for IL6. Significant main effect of diabetes was found in IL6 ($P = 0.006$). The protein abundance of IL6 was significantly increased by 37% ($P = 0.003$) in muscle of db/dbCON mice relative to that of db/+CON mice (**Figure 4A**). Significant interaction effect of diabetes and DOX was observed for TNF α ($P = 0.038$). The protein abundance of TNF α was increased by 35% ($P = 0.0001$) in muscle of db/dbDOX mice relative to that of db/+DOX. TNF α was also increased by 13% ($P = 0.036$) in the muscle of db/dbDOX mice relative to db/dbCON mice (**Figure 4B**). No interaction effect of diabetes and DOX was observed for NFkB. Significant main effect of diabetes was observed for NFkB ($P = 0.001$). There was a significant 47% ($P = 0.001$) increase in phosphor-NFkB in db/dbDOX muscle relative to db/+DOX muscle and a significant 46% ($P = 0.0023$) increase in phosphor-NFkB in db/dbDOX muscle relative to db/dbCON muscle (**Figure 4C**). Significant interaction effect of diabetes with DOX was observed in HIF-1 α ($P = 0.05$). The protein abundance of HIF-1 α was increased by 635% ($P = 0.0001$) in muscle of db/dbDOX mice relative to db/+DOX mice. HIF-1 α and increased by 47% ($P = 0.05$) in db/dbDOX mice relative to that of db/dbCON mice (**Figure 4D**).

DOX Caused a Shift toward Anaerobic Glycolysis in Diabetic Muscle

Anaerobic glycolysis favoring metabolic regulators including pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4), ACC, and LDH were examined. Significant interaction effect



of diabetes with DOX was observed in PDK4 ($P = 0.019$). The protein abundance of PDK4 significantly increased by 94% ($P = 0.03$) in the muscle of db/dbDOX mice relative to the muscle of db/+DOX mice and significantly increased by 34% ($P = 0.05$) in the muscle of db/dbDOX mice relative to that of db/dbCON mice (Figure 5A). Significant interaction effect of diabetes with DOX was observed in ACC ($P = 0.007$). There was a significant 12% ($P = 0.045$) decrease in protein abundance of phosphor-ACC in the muscle of db/dbCON relative to that of db/+CON mice. Muscle of db/dbDOX mice showed a significant 68% ($P = 0.02$) decrease in protein

abundance of phosphor-ACC relative to that of db/dbCON mice (Figure 5B). Significant interaction effect of diabetes and DOX was observed for both LDH protein abundance ($P = 0.0001$) and LDH activity ($P = 0.047$). There was a significant 47% ($P = 0.0001$) increase in the protein abundance of LDH in the muscle of db/dbDOX mice relative to db/+DOX mice and a 31% ($P = 0.01$) increase in LDH in the muscle of db/dbDOX mice relative to that of db/dbCON (Figure 5C). A significant 134% ($P = 0.001$) increase in LDH activity in muscle of db/dbDOX mice relative to that of db/+DOX mice was found (Figure 5D).

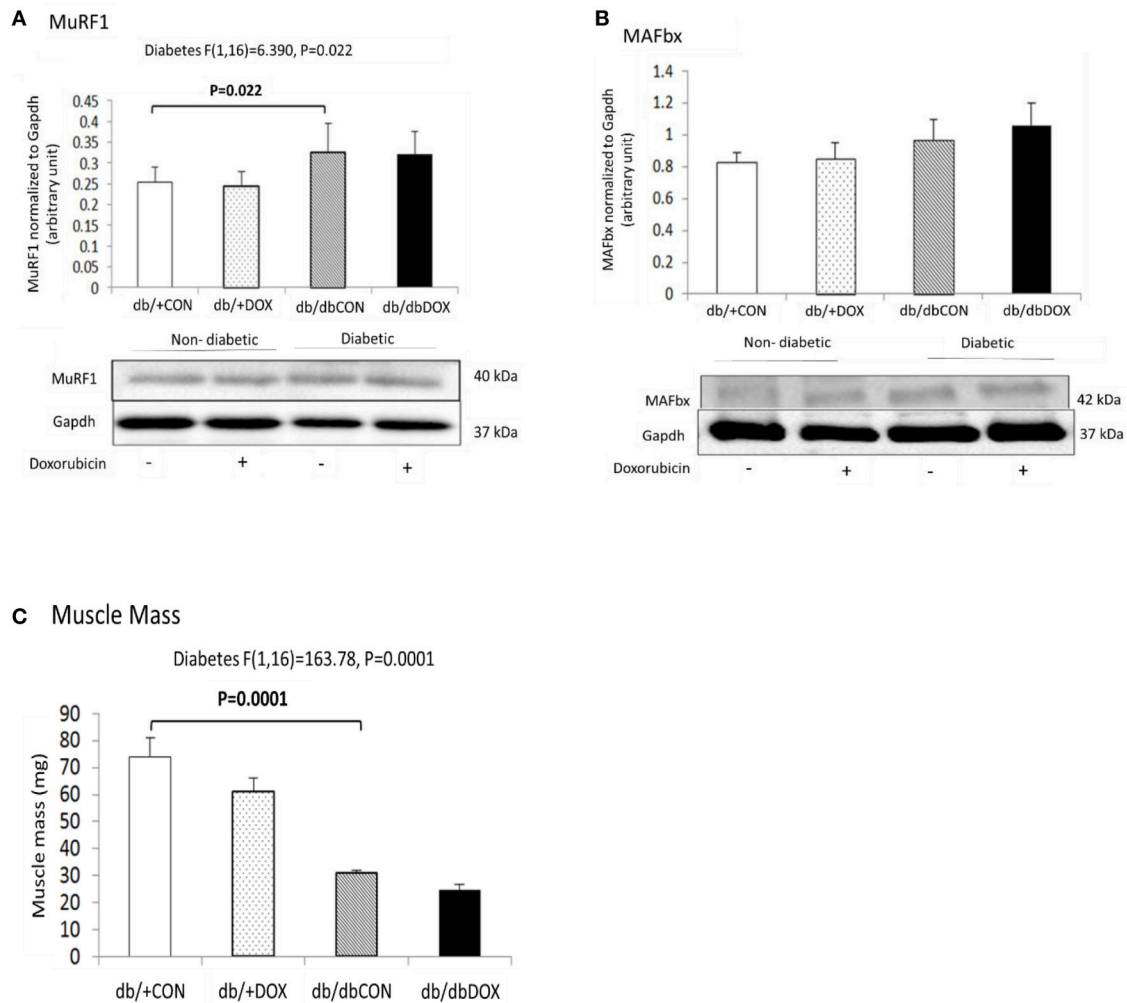


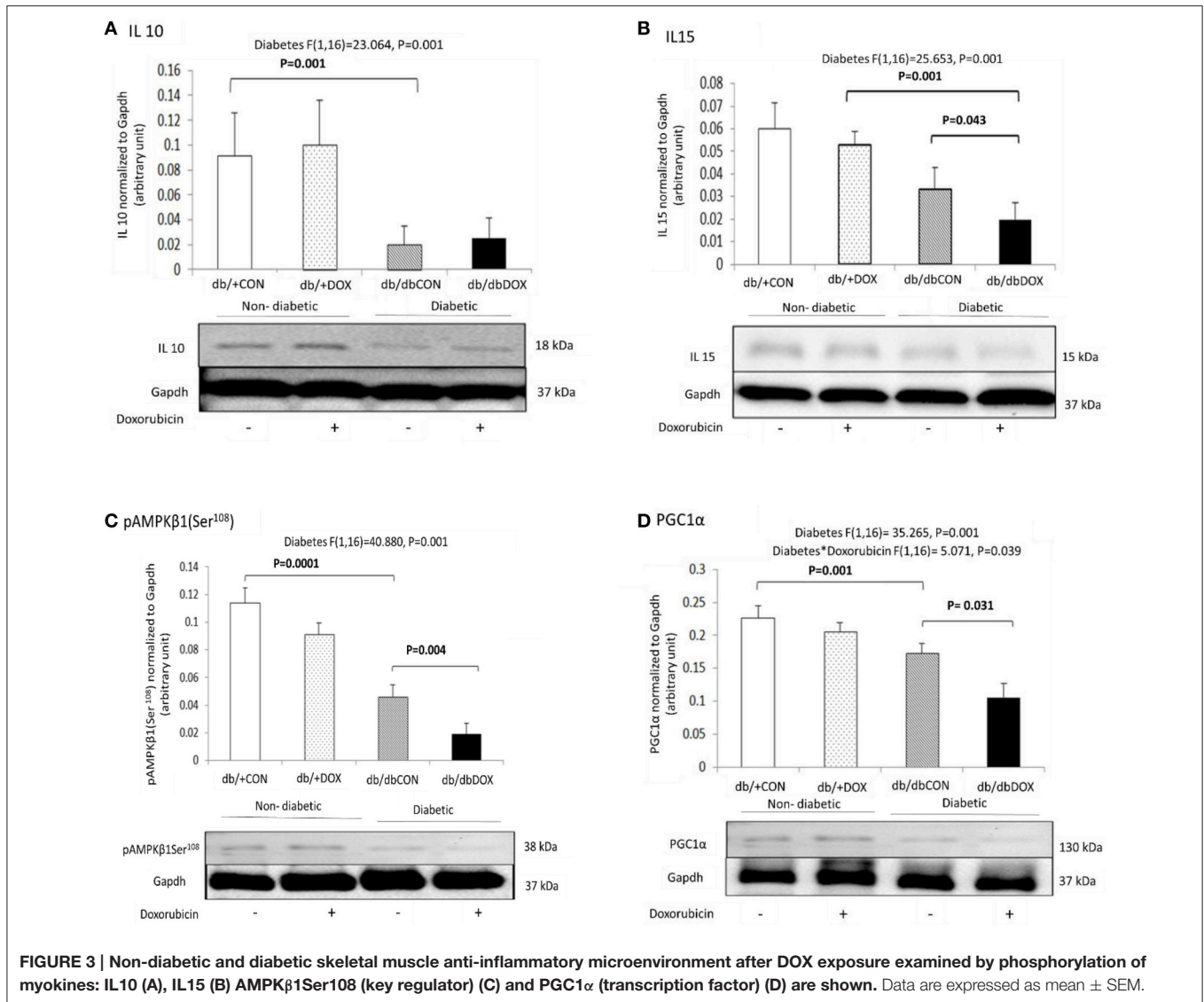
FIGURE 2 | Non-diabetic and diabetic skeletal muscle atrophy after DOX exposure examined by phosphorylation statuses of MuRF1 (A) and MAFbx (B) muscle mass (mg) (C) are shown. Data are expressed as mean \pm SEM.

DISCUSSION

In this study, we demonstrated that DOX treatment in diabetic skeletal muscle did not exacerbate the impairment of insulin signaling and muscle atrophy as compared to non-diabetic skeletal muscle. However, DOX augmented pro-inflammatory microenvironment in diabetic muscle by upregulating transcription factor HIF1 α , regulatory molecule pNF κ Bp65, and cytokine TNF α . DOX also altered anti-inflammatory microenvironment by downregulating transcription factor PGC1 α , regulatory molecule pAMPK β 1Ser108 and muscle-specific myokine IL15. In addition, DOX induced dysregulated glycolytic metabolism in diabetic skeletal muscle by upregulating PDK4 and LDH and downregulating phosphorylation of acetyl-CoA carboxylase (pACC Ser79).

Type 2 diabetes (T2D) is a progressive chronic disease, primarily characterized by functional decline of beta cell,

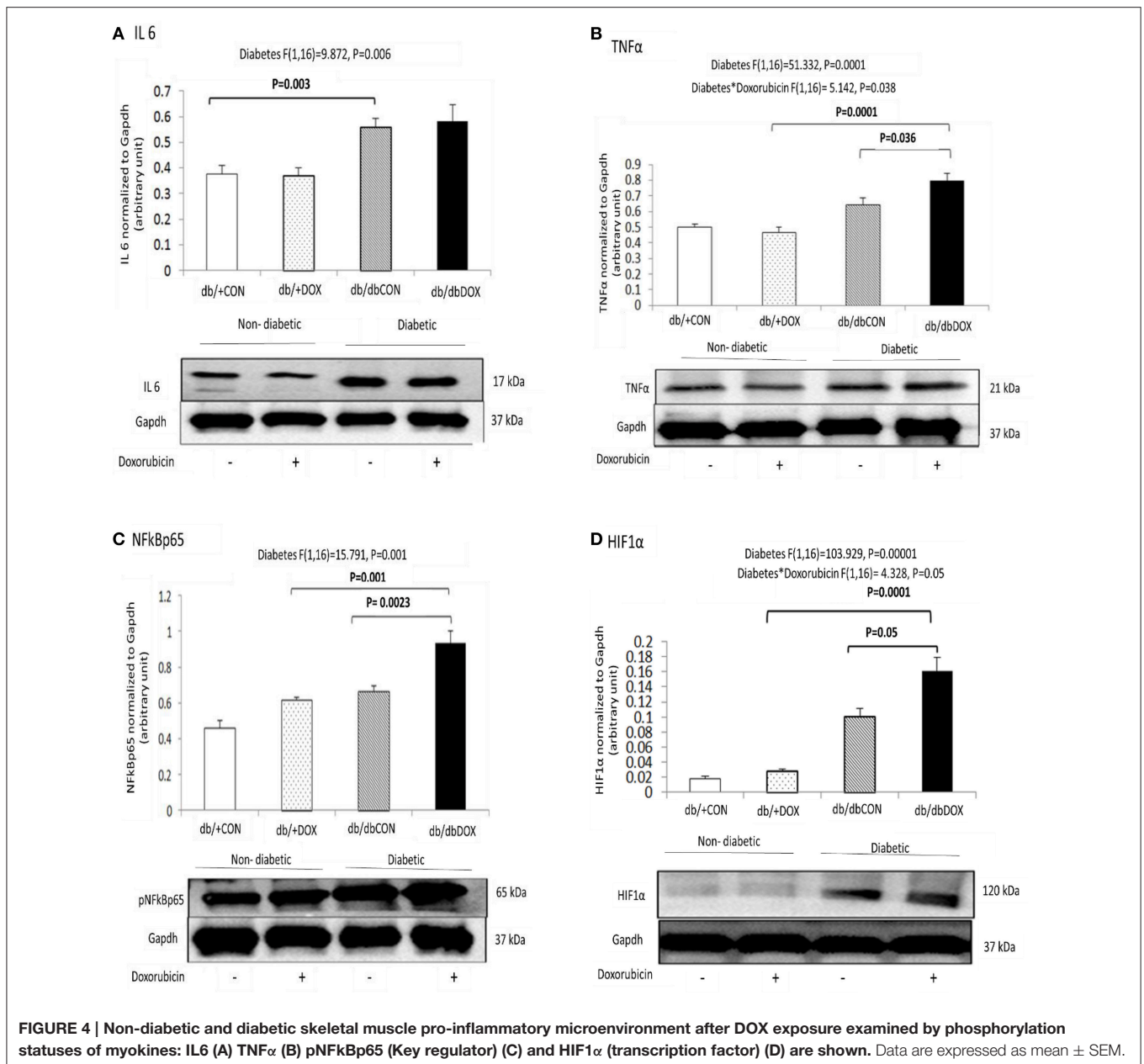
worsening of insulin signaling, and muscle atrophy (Trostler et al., 1982; Cotter et al., 1993; Fonseca, 2009; Brannmark et al., 2013; de Lima Junior et al., 2016). DOX administration in non-diabetic skeletal muscle has been shown to cause insulin resistance and degradation of muscle quality (Hydock et al., 2011; Smuder et al., 2011; de Lima Junior et al., 2016). In the present study, our results are consistent with the previous findings of insulin signaling and muscle atrophy markers in diabetic muscle and DOX-treated muscle. However, our data indicated that there was no significant difference in insulin signaling and muscle atrophy markers in DOX-treated diabetic skeletal muscles when compared to diabetic skeletal muscles without DOX exposure. We interpreted that, in diabetic skeletal muscle, the diabetic characteristics (i.e., insulin signaling and muscle atrophy) were already worsened and hence the DOX exposure did not lead to a further impairment of insulin signaling and muscle atrophy markers. We did not observe significant difference in anti-inflammatory microenvironment (IL10, IL15,



AMPK, and PGC1 α), pro-inflammatory microenvironment (IL 6, TNF α , NF κ B, and HIF1 α) as well as in metabolic regulators (ACC, PDK4, and LDH) in skeletal muscles treated with DOX when compared to skeletal muscle without DOX exposure. We inferred that, DOX exposure may primarily affect the insulin sensitivity (de Lima Junior et al., 2016) and muscle atrophy (Chen et al., 2007) due to the accumulation of reactive oxygen species in the skeletal muscle (van Etten et al., 1998; Hardin et al., 2008; van Norren et al., 2009), and this is affected by the modulation of inflammatory microenvironment and metabolic dysregulation in the plasma (Haus et al., 2009). Nevertheless, our results suggested that DOX administration in diabetic skeletal muscle might cause an environment which is favorable toward aggregating pathogenesis of T2D as indicated by our observations of the inflammatory upsurge and metabolic dysregulation (Rhodes, 2005; Fonseca, 2009; Rocha et al., 2016).

The Link of Diminution of Anti-Inflammatory Microenvironment and Dysregulated Oxidative Metabolism

AMPK, a regulatory molecule favoring anti-inflammatory microenvironment (O'Neill and Hardie, 2013), directly activates PGC1 α (Jäger et al., 2007) and regulates IL15 (Crane et al., 2015). Therefore, downregulation of AMPK in diabetic skeletal muscles after DOX treatment leading to the reduction of PGC1 α and IL15 might indicate the diminution of the anti-inflammatory microenvironment, as shown in our results. AMPK activation in skeletal muscle has been shown to result in muscle fiber shifting from glycolytic fibers to oxidative fibers in a PGC1 α -dependent manner (Garcia-Roves et al., 2008). AMPK was also demonstrated to stimulate fatty acid oxidation and reduce the activity of acetyl-coenzyme A (CoA) carboxylase, which is an enzyme carrying out the conversion of acetyl CoA to malonyl CoA (Castle et al., 2009) that directs fatty acid



oxidation in skeletal muscle (Abu-Elheiga et al., 2001). Since the phosphorylation of ACC was significantly downregulated in our examined muscles, we suspected that DOX decreased fatty acid oxidative metabolism in diabetic skeletal muscle. It has been reported that oxidative phosphorylation (OXPHOS) genes are strongly correlated to PGC1 α in diabetic skeletal muscle based on DNA microarray analysis (Mootha et al., 2003); also, it has been reported that DOX treatment in the heart inhibited oxidative phosphorylation (Abdel-aleem et al., 1997) and reduced phosphorylation of ACC in white adipose tissue (Biondo et al., 2016). Consistently, we demonstrated in our examined muscle, the decreases in AMPK and PGC1 α , which induced glycolytic shift as indicated by the downregulation of pACCSer79. Overall, our findings suggest that there might

be a link between anti-inflammatory microenvironment and dysregulated metabolism in the DOX-induced myotoxicity in diabetic skeletal muscle. Further research is needed to fully dissect the regulatory mechanisms of the link the inflammatory microenvironment and metabolism dysregulation in DOX-injured diabetic skeletal muscle.

The Link of Augmentation of Pro-Inflammatory Microenvironment and Anaerobic Glycolytic Metabolism

NF κ B, a regulatory molecule favoring pro-inflammatory microenvironment (Eisele et al., 2013) has been shown to be activated by ROS (Dodd et al., 2010) and TNF α (Remels

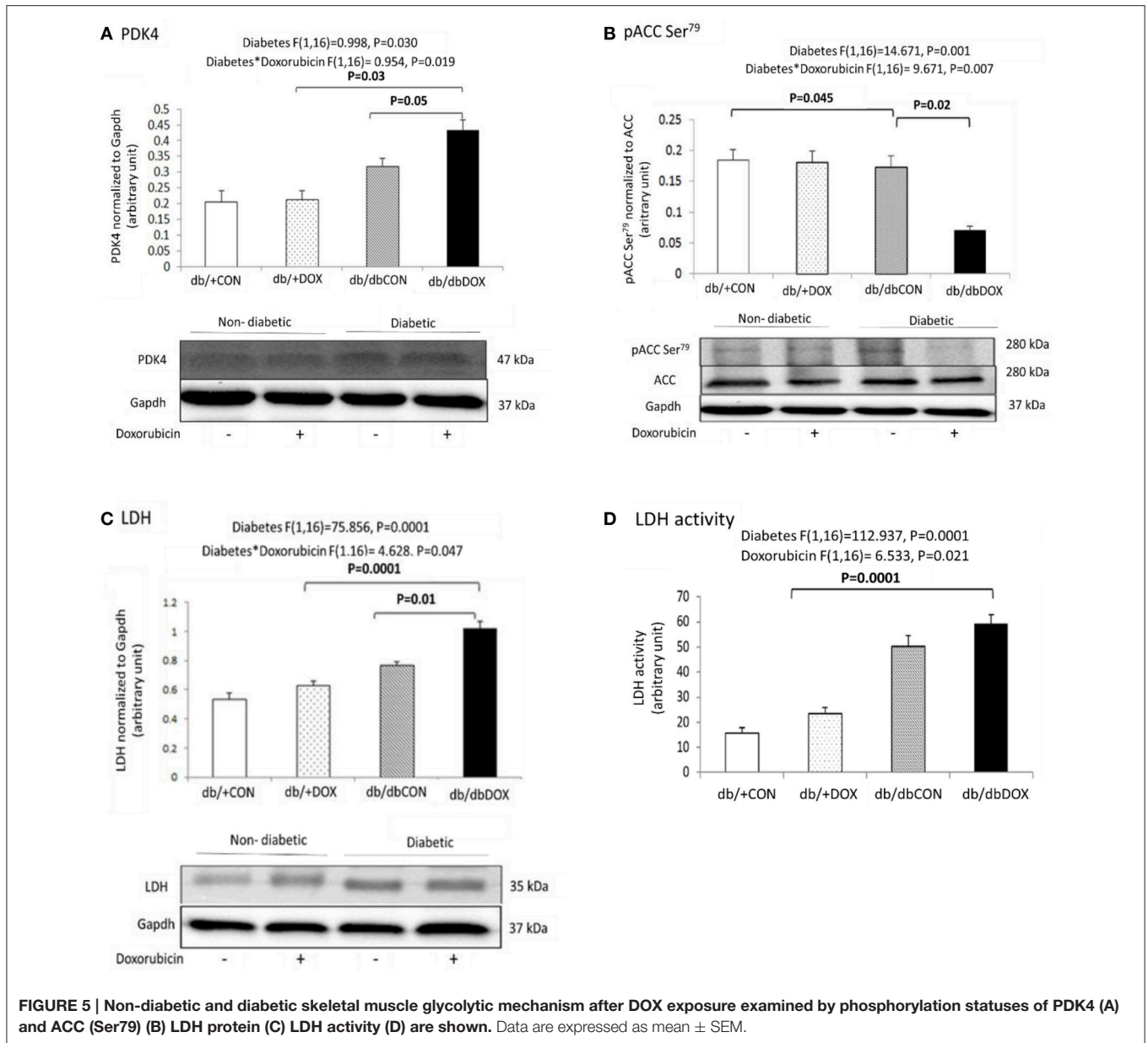
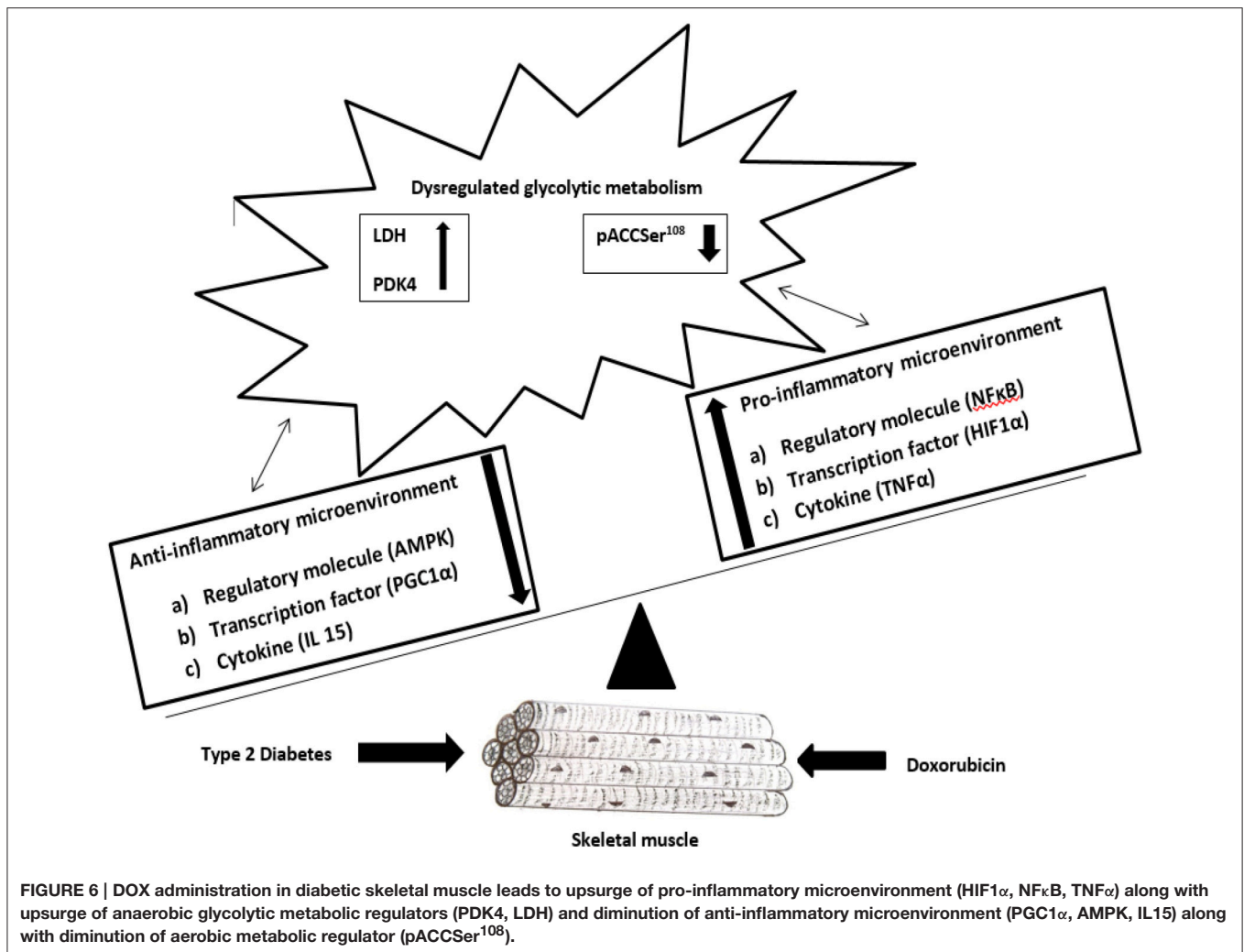


FIGURE 5 | Non-diabetic and diabetic skeletal muscle glycolytic mechanism after DOX exposure examined by phosphorylation statuses of PDK4 (A) and ACC (Ser79) (B) LDH protein (C) LDH activity (D) are shown. Data are expressed as mean \pm SEM.

et al., 2014) in skeletal muscle. Additionally, classical activation of NF κ B was demonstrated to augment muscle glycolytic metabolism in a HIF1 α -dependent manner (Remels et al., 2014). Our results exhibited that the upregulation of TNF α activated NF κ B and augmented glycolytic metabolism in HIF1 α -dependent manner. Notably, DOX has been reported to induce cardiotoxicity by decreasing conversion of acetyl-CoA to malonyl CoA and the decreased rate of conversion inhibited fatty acid oxidation and ATP generation (Peluso et al., 2000). Consistently, an anti-diabetic drug (metformin) has been shown to prevent cardiotoxicity induced by DOX by increasing fatty acid oxidation and preventing energy starvation (Ashour et al., 2012). Metformin in combination with DOX has been demonstrated to inhibit the inflammatory

pathway by inhibiting NF κ B (a pro-inflammatory regulatory molecule) in mammalian cancer cell line (Hirsch et al., 2013). We suspected that if metformin suppressed the effect of DOX toxicity by enhancing phosphorylation of ACC and decreasing NF κ B, then DOX induction along with diabetes might reduce ACC phosphorylation and aggravate inflammation by triggering NF κ B according to the present findings. Since NF κ B augmented muscle glycolytic metabolism in a HIF1 α -dependent manner (Remels et al., 2014), HIF1 α upregulation might be an indicator of an increase in glycolytic metabolism, as shown in our data. Our observed increase in TNF α suggested that NF κ B was increased in a PGC1 α -dependent fashion as it has been demonstrated that TNF α increased NF κ B binding to PGC1 α , which downregulated PGC1 α and subsequently



dysregulated glucose metabolism (Lvarez-Guardia et al., 2010).

The Upsurge of Anaerobic Glycolytic Metabolism

Diabetes causes muscle energy starvation (Simoneau and Kelley, 1997) and DOX causes muscle fatigue (van Norren et al., 2009); hence, it is proposed that when DOX is administered in diabetic skeletal muscle, pro-inflammatory microenvironment upsurge might take place to undergo profound metabolic changes to mediate metabolic adaptation caused by DOX in diabetic skeletal muscle. It has been shown that HIF1 α (pro-inflammatory transcription factor) led to upregulation of anaerobic glycolysis and release of lactate (Tannahill et al., 2013). Glycolysis is the metabolic pathway that converts glucose to pyruvate aerobically and lactate anaerobically (Scott, 2008). Interconversion of pyruvate to lactate and vice versa is carried out by LDH during intensive exercise (Spriet, 1995). Consistently, we observed increases in HIF1 α and LDH in our examined muscles and it might be possible that the upsurge of pro-inflammatory regulatory molecule has stabilized HIF1 α , which

led to lactate release posing an aerobic-to-anaerobic metabolic shift. Our interpretation is supported by a study reporting that diabetic skeletal muscle has increased glycolytic rate with upregulation of LDH (Tannahill et al., 2013). The increase in pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4) indicated the accumulation of fatty acid, which has been demonstrated to be upregulated in diabetic skeletal muscle (Rosa et al., 2003) and in DOX-treated skeletal muscle (Sin et al., 2015). Nonetheless, HIF1 α (pro-inflammatory transcription factor) might also have induced the alteration of PDK4 (Meiser et al., 2016), which was consistent with our findings. Collectively, our results demonstrated that DOX increased the abundances of PDK4, LDH and HIF1 α with the downregulation of ACC phosphorylation, and established a pro-inflammatory environment in diabetic skeletal muscle in a HIF1 α -dependent manner.

DOX and diabetes individually have been demonstrated to cause the release of ROS that might lead to muscle fatigue and metabolic dysregulation (van Etten et al., 1998; Hardin et al., 2008; van Norren et al., 2009; Ritov et al., 2010), which are in accordance with our present findings of muscle

atrophy and insulin signaling markers. Hence, we speculated that DOX in diabetic skeletal muscle might have induced further elevation of ROS production when compared to DOX or diabetes alone. Excessive ROS production has been shown to increase the inflammatory response (Barreiro et al., 2005; Fruehauf and Meyskens, 2007; Zuo et al., 2013a,b) and dysregulate multiple regulatory enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (Zuo et al., 2015). In DOX-treated diabetic skeletal muscle, we observed the upregulation of pro-inflammatory microenvironment regulatory molecule (NF κ B) and cytokines (TNF α) that might be due to the over-production of ROS (Wang et al., 2011). The balance of ROS has been shown to play an important role in the adaptation and response of the glycolytic activity (Blair et al., 1999). In the present study, the observations on the increase in LDH and PDK4 along with the decrease in ACC leading to dysregulated metabolism suggest that there might be excessive ROS production in DOX-treated diabetic skeletal muscle. High level of glucose or fatty acids is known to enhance ROS production, which plays a vital role in the loss of the number or function of pancreatic beta cells. On the contrary, ROS have been reported to be produced under high or low glucose concentration in pancreatic beta cells by activating AMPK in a superoxide-dependent manner (Sarre et al., 2012). In our results, we found that AMPK was downregulated suggesting that high level of glucose might have resulted in the over-production of ROS in the DOX-treated diabetic skeletal muscle. As ROS play a critical role in inflammation, calcium flux, muscle death and atrophy, diabetes and aerobic metabolism, additional investigation is warranted to comprehensively examine the role of ROS in the modulations of inflammation and metabolism in DOX-treated diabetic muscle.

In conclusion, our data demonstrated that the modulation of the inflammatory pathway might be linked to the shift of oxidative-glycolytic metabolism in diabetic skeletal muscle after DOX exposure (Figure 6). Our findings that the increases in

pro-inflammatory transcription factor HIF1 α and regulatory molecule NF κ B along with the augmentation of inflammatory cytokine TNF α in DOX-injured diabetic muscle might help to reveal the underlying mechanisms and thus hasten the development of new effective strategies to protect skeletal muscle from DOX-induced toxicity in diabetic cancer patients. Moreover, the present findings of the decreases in transcription factor PGC1 α and regulatory molecule AMPK along with the diminution of anti-inflammatory myokine IL15 in DOX-injured diabetic muscle might indicate inverse relationship with anti-inflammatory microenvironment and glycolytic metabolism. Although the adopted sample size has resulted in a statistical power of 80% or above for the assessments on muscle mass, IRS1, MAFbx, IL6, IL10, IL15, AMPK, PGC1 α , HIF1 α , and LDH activity, the constraint of sample size might be a limitation in the present study. Furthermore, additional research is warranted to further investigate the toxic effects of DOX on skeletal muscle in a tumor diabetic animal model.

AUTHOR CONTRIBUTIONS

RS, BT, XP, and PS designed the studies; RS, BT, and XP performed the experiments; CL, LC, and BY contributed to discussion and editing; RS, BY, and PS analyzed and interpreted data, supervised the project and co-wrote the manuscript.

ACKNOWLEDGMENTS

This study was supported by the Hong Kong Research Grants Council Hong Kong PhD Fellowship Scheme (RTVX PF13-11753) and The Hong Kong Polytechnic University Research Fund (G-YN48, G-YBBH, G-YBFX, and 1-ZE17). The authors acknowledge the animal husbandry support received from the Centralized Animal Facilities of The Hong Kong Polytechnic University.

REFERENCES

- Abdel-aleem, S., El-Merzabani, M. M., Sayed-Ahmed, M., Taylor, D. A., and Lowe, J. E. (1997). Acute and chronic effects of adriamycin on fatty acid oxidation in isolated cardiac myocytes. *J. Mol. Cell. Cardiol.* 29, 789–797. doi: 10.1006/jmcc.1996.0323
- Abu-Elheiga, L., Matzuk, M. M., Abo-Hashema, K. A., and Wakil, S. J. (2001). Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science (New York, N.Y.)* 291, 2613–2616. doi: 10.1126/science.1056843
- Aluise, C. D., Sultana, R., Tangpong, J., Vore, M., St Clair, D., Moscow, J. A., et al. (2010). Chemo brain (chemo fog) as a potential side effect of DOX administration: role of cytokine-induced, oxidative/nitrosative stress in cognitive dysfunction. *Adv. Exp. Med. Biol.* 678, 147–156. doi: 10.1007/978-1-4419-6306-2_19
- Arthur, P. G., Grounds, M. D., and Shavlakadze, T. (2008). Oxidative stress as a therapeutic target during muscle wasting: considering the complex interactions. *Curr. Opin. Clin. Nutr. Metab. Care* 11, 408–416. doi: 10.1097/MCO.0b013e328302f3fe
- Ashour, A. E., Sayed-Ahmed, M. M., Abd-Allah, A. R., Korashy, H. M., Maayah, Z. H., Alkhalidi, H., et al. (2012). Metformin rescues the Myocardium from DOX-induced energy starvation and mitochondrial damage in rats. *Oxid. Med. Cell. Longev.* 2012, 1–13. doi: 10.1155/2012/434195
- Barreiro, E., de la Puente, B., Minguella, J., Corominas, J. M., Serrano, S., Hussain, S. N., et al. (2005). Oxidative stress and respiratory muscle dysfunction in severe chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 171, 1116–1124. doi: 10.1164/rccm.200407-887OC
- Biondo, L. A., Lima Junior, E. A., Souza, C. O., Cruz, M. M., Cunha, R. D. C., Alonso-Vale, M. I., et al. (2016). Impact of DOX treatment on the physiological functions of white adipose tissue. *PLoS ONE* 11:e0151548. doi: 10.1371/journal.pone.0151548
- Blair, A. S., Hajdich, E., Litherland, G. J., and Hundal, H. S. (1999). Regulation of glucose transport and glycogen synthesis in L6 muscle cells during oxidative stress. Evidence for cross-talk between the insulin and SAPK2/p38 mitogen-activated protein kinase signaling pathways. *J. Biol. Chem.* 274, 36293–36299. doi: 10.1074/jbc.274.51.36293
- Brannmark, C., Nyman, E., Fagerholm, S., Bergenholm, L., Ekstrand, E.-M., Cedersund, G., et al. (2013). Insulin Signaling in Type 2 Diabetes: EXPERIMENTAL AND MODELING ANALYSES REVEAL MECHANISMS OF INSULIN RESISTANCE IN HUMAN ADIPOCYTES. *J. Biol. Chem.* 288, 9867–9880. doi: 10.1074/jbc.M112.432062

- Braun, T. P., Szumowski, M., Levasseur, P. R., Grossberg, A. J., Zhu, X., Agarwal, A., et al. (2014). Muscle atrophy in response to cytotoxic chemotherapy is dependent on intact glucocorticoid signaling in skeletal muscle. *PLoS ONE* 9:e106489. doi: 10.1371/journal.pone.0106489
- Castle, J. C., Hara, Y., Raymond, C. K., Garrett-Engele, P., Ohwaki, K., Kan, Z., et al. (2009). ACC2 is expressed at high levels in human white adipose and has an isoform with a novel N-terminus [corrected]. *PLoS ONE* 4:e4369. doi: 10.1371/journal.pone.0004369
- Chen, Y., Jungsuwadee, P., Vore, M., Butterfield, D. A., and St Clair, D. K. (2007). Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. *Mol. Interv.* 7, 147–156. doi: 10.1124/mi.7.3.6
- Cortés-Funes, H., and Coronado, C. (2007). Role of anthracyclines in the era of targeted therapy. *Cardiovasc. Toxicol.* 7, 56–60. doi: 10.1007/s12012-007-0015-3
- Cotter, M. A., Cameron, N. E., Robertson, S., and Ewing, I. (1993). Polyol pathway-related skeletal muscle contractile and morphological abnormalities in diabetic rats. *Exp. Physiol.* 78, 139–155. doi: 10.1113/expphysiol.1993.sp003675
- Crane, J. D., MacNeil, L. G., Lally, J. S., Ford, R. J., Bujak, A. L., Brar, I. K., et al. (2015). Exercise-stimulated interleukin-15 is controlled by AMPK and regulates skin metabolism and aging. *Aging Cell* 14, 625–634. doi: 10.1111/acel.12341
- de Lima Junior, E. A., Yamashita, A. S., Pimentel, G. D., De Sousa, L. G. O., Santos, R. V. T., Gonçalves, C. L., et al. (2016). Doxorubicin caused severe hyperglycaemia and insulin resistance, mediated by inhibition in AMPK signalling in skeletal muscle. *J. Cachexia Sarcopenia Muscle*. doi: 10.1002/jcsm.12104. [Epub ahead of print].
- Dodd, S. L., Gagnon, B. J., Senf, S. M., Hain, B. A., and Judge, A. R. (2010). Ros-mediated activation of NF- κ B and Foxo during muscle disuse. *Muscle Nerve* 41, 110–113. doi: 10.1002/mus.21526
- Eisele, P. S., Salatino, S., Sobek, J., Hottiger, M. O., and Handschin, C. (2013). The peroxisome proliferator-activated receptor γ coactivator 1 α/β (PGC-1) coactivators repress the transcriptional activity of NF- κ B in skeletal muscle cells. *J. Biol. Chem.* 288, 2246–2260. doi: 10.1074/jbc.M112.375253
- Fabris, S., and MacLean, D. A. (2015). Skeletal muscle an active compartment in the sequestering and metabolism of DOX chemotherapy. *PLoS ONE* 10:e0139070. doi: 10.1371/journal.pone.0139070
- Falkenberg, J. H., Iaizzo, P. A., and McLoon, L. K. (2001). Physiological assessment of muscle strength in vitro after direct injection of DOX into rabbit sternocleidomastoid muscle. *Movem. Disord.* 16, 683–692. doi: 10.1002/mds.1125
- Fonseca, V. A. (2009). Defining and characterizing the progression of type 2 diabetes. *Diabetes Care* 32, S151–S156. doi: 10.2337/dc09-S301
- Franckhauser, S., Elias, L., Rotter Sopasakis, V., Ferré, T., Nagaev, I., Andersson, C. X., et al. (2008). Overexpression of Il6 leads to hyperinsulinaemia, liver inflammation and reduced body weight in mice. *Diabetologia* 51, 1306–1316. doi: 10.1007/s00125-008-0998-8
- Fruehauf, J. P., and Meyskens, F. L. (2007). Reactive oxygen species: a breath of life or death? *Clin. Cancer Res.* 13, 789–794. doi: 10.1158/1078-0432.ccr-06-2082
- Garcia-Roves, P. M., Osler, M. E., Holmstrom, M. H., and Zierath, J. R. (2008). Gain-of-function R225Q mutation in AMP-activated protein kinase 3 subunit increases mitochondrial biogenesis in glycolytic skeletal muscle. *J. Biol. Chem.* 283, 35724–35734. doi: 10.1074/jbc.M805078200
- Gilliam, L. A. A., and St. Clair, D. K. (2011). Chemotherapy-induced weakness and fatigue in skeletal muscle: the role of oxidative stress. *Antiox. Redox Signal.* 15, 2543–2563. doi: 10.1089/ars.2011.3965
- Gokcimen, A., Cim, A., Tola, H. T., Bayram, D., Kocak, A., OZguner, F., et al. (2007). Protective effect of N-acetylcysteine, caffeic acid and vitamin E on DOX hepatotoxicity. *Hum. Exp. Toxicol.* 26, 519–525. doi: 10.1177/0960327107076885
- Grimberg, A. (2003). Mechanisms by which IGF-I may promote cancer. *Cancer Biol. Ther.* 2, 630–635. doi: 10.4161/cbt.2.6.678
- Hardin, B. J., Campbell, K. S., Smith, J. D., Arbogast, S., Smith, J., Moylan, J. S., et al. (2008). TNF- α acts via TNFR1 and muscle-derived oxidants to depress myofibrillar force in murine skeletal muscle. *J. Appl. Physiol.* 104, 694–699. doi: 10.1152/jappphysiol.00898.2007
- Haus, J. M., Kashyap, S. R., Kasumov, T., Zhang, R., Kelly, K. R., DeFronzo, R. A., et al. (2009). Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* 58, 337–343. doi: 10.2337/db08-1228
- Hayward, R., Hydock, D., Gibson, N., Greufe, S., Bredahl, E., and Parry, T. (2013). Tissue retention of DOX and its effects on cardiac, smooth, and skeletal muscle function. *J. Physiol. Biochem.* 69, 177–187. doi: 10.1007/s13105-012-0200-0
- Hirsch, H. A., Iliopoulos, D., and Struhl, K. (2013). Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proc. Natl. Acad. Sci. U.S.A.* 110, 972–977. doi: 10.1073/pnas.1221055110
- Hotamisligil, G. S., and Spiegelman, B. M. (1994). Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes* 43, 1271–1278. doi: 10.2337/diab.43.11.1271
- Hydock, D. S., Lien, C.-Y., Jensen, B. T., Schneider, C. M., and Hayward, R. (2011). Characterization of the effect of in vivo DOX treatment on skeletal muscle function in the rat. *Anticancer Res.* 31, 2023–2028.
- Irrcher, I., Ljubicic, V., and Hood, D. A. (2008). Interactions between ROS and AMP kinase activity in the regulation of PGC-1 transcription in skeletal muscle cells. *AJP Cell Physiol.* 296, C116–C123. doi: 10.1152/ajpcell.00267.2007
- Jäger, S., Handschin, C., St-Pierre, J., and Spiegelman, B. M. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 α . *Proc. Natl. Acad. Sci. U.S.A.* 104, 12017–12022. doi: 10.1073/pnas.0705070104
- Liao, J. K. (2012). Mitohormesis: another pleiotropic effect of statins? *Eur. Heart J.* 33, 1299–1301. doi: 10.1093/eurheartj/ehr287
- Lim, K.-H., Yoon, H.-I., Kang, Y. A., Lee, K.-W., Kim, J. H., Bang, S.-M., et al. (2010). Severe pulmonary adverse effects in lymphoma patients treated with cyclophosphamide, DOX, vincristine, and prednisone (CHOP) regimen plus rituximab. *Korean J. Intern. Med.* 25, 86–92. doi: 10.3904/kjim.2010.25.1.86
- Lvarez-Guardia, D., Palomer, X., Coll, T., Davidson, M. M., Chan, T. O., Feldman, A. M., et al. (2010). The p65 subunit of NF- κ B binds to PGC-1, linking inflammation and metabolic disturbances in cardiac cells. *Cardiovasc. Res.* 87, 449–458. doi: 10.1093/cvr/cvq080
- Martins, A. R., Nachbar, R. T., Goro, R., Vinolo, M. A., Festuccia, W. T., Lambertucci, R. H., et al. (2012). Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function. *Lipids Health Dis.* 11, 1–11. doi: 10.1186/1476-511X-11-30
- Meiser, J., Krämer, L., Sapcariu, S. C., Battello, N., Ghelfi, J., D'Herouel, A. F., et al. (2016). Pro-inflammatory macrophages sustain pyruvate oxidation through pyruvate dehydrogenase for the synthesis of itaconate and to enable cytokine expression. *J. Bio. Chem.* 291, 3932–3946. doi: 10.1074/jbc.M115.676817
- Min, K., Kwon, O.-S., Smuder, A. J., Wiggs, M. P., Sollanek, K. J., Christou, D. D., et al. (2015). Increased mitochondrial emission of reactive oxygen species and calpain activation are required for DOX-induced cardiac and skeletal muscle myopathy. *J. Physiol. (Lond.)* 593, 2017–2036. doi: 10.1113/jphysiol.2014.286518
- Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., and Gianni, L. (2004). Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev.* 56, 185–229. doi: 10.1124/pr.56.2.6
- Mootha, V. K., Lindgren, C. M., Eriksson, K.-F., Subramanian, A., Sihag, S., Lehar, J., et al. (2003). PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* 34, 267–273. doi: 10.1038/ng1180
- Murata, T., Yamawaki, H., Hori, M., Sato, K., Ozaki, H., and Karaki, H. (2001). Chronic vascular toxicity of DOX in an organ-cultured artery. *Br. J. Pharmacol.* 132, 1365–1373. doi: 10.1038/sj.bjp.0703959
- Nitiss, J. L. (2009). Targeting DNA topoisomerase II in cancer chemotherapy. *Nat. Rev. Cancer* 9, 338–350. doi: 10.1038/nrc2607
- O'Neill, L. A. J., and Hardie, D. G. (2013). Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* 493, 346–355. doi: 10.1038/nature11862
- Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., and Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J. Physiol. (Lond.)* 515, 287–291. doi: 10.1111/j.1469-7793.1999.287ad.x
- Pei, X. M., Yung, B. Y., Yip, S. P., Chan, L. W., Wong, C. S., Ying, M., et al. (2015). Protective effects of desacyl ghrelin on diabetic cardiomyopathy. *Acta Diabetol.* 52, 293–306. doi: 10.1007/s00592-014-0637-4

- Peluso, G., Nicolai, R., Reda, E., Benatti, P., Barbarisi, A., and Calvani, M. (2000). Cancer and anticancer therapy-induced modifications on metabolism mediated by carnitine system. *J. Cell. Physiol.* 182, 339–350. doi: 10.1002/(SICI)1097-4652(200003)182:3<339::AID-JCP4>3.0.CO;2-B
- Pickup, J. (2004). Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* 27, 813–823. doi: 10.2337/diacare.27.3.813
- Psarakis, H. M. (2006). Clinical challenges in caring for patients with diabetes and cancer. *Diabetes Spect.* 19, 157–162. doi: 10.2337/diaspect.19.3.157
- Remels, A. H. V., Gosker, H. R., Verhees, K. J. P., Langen, R. C. J., and Schols, A. M. W. J. (2014). TNF- α -induced NF- κ B activation stimulates skeletal muscle glycolytic metabolism through activation of HIF-1 α . *Endocrinology* 156, 1770–1781. doi: 10.1210/en.2014-1591
- Rhodes, C. J. (2005). Type 2 diabetes—a matter of beta-cell life and death? *Science (New York, N.Y.)* 307, 380–384. doi: 10.1126/science.1104345
- Richardson, L. C., and Pollack, L. A. (2005). Therapy Insight: influence of type 2 diabetes on the development, treatment and outcomes of cancer. *Nat. Clin. Pract. Oncol.* 2, 48–53. doi: 10.1038/ncponc0062
- Ritov, V. B., Menshikova, E. V., Azuma, K., Wood, R., Toledo, F. G. S., Goodpaster, B. H., et al. (2010). Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. *AJP Endocrinol. Metab.* 298, E49–E58. doi: 10.1152/ajpendo.00317.2009
- Rocha, M., Diaz-Morales, N., Rovira-Llopis, S., Escribano-Lopez, I., Bañuls, C., Hernandez-Mijares, A., et al. (2016). Mitochondrial dysfunction and endoplasmic reticulum stress in diabetes. *Curr. Pharm. Des.* 22, 2640–2649. doi: 10.2174/1381612822666160209152033
- Rosa, G., Di Rocco, P., Manco, M., Greco, A. V., Castagneto, M., Vidal, H., et al. (2003). Reduced PDK4 expression associates with increased insulin sensitivity in postobese patients. *Obes. Res.* 11, 176–182. doi: 10.1038/oby.2003.28
- Sag, D., Carling, D., Stout, R. D., and Suttles, J. (2008). AMP-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. *J. Immunol.* 181, 8633–8641. doi: 10.4049/jimmunol.181.12.8633
- Sarre, A., Gabrielli, J., Vial, G., Leverve, X. M., and Assimacopoulos-Jeannet, F. (2012). Reactive oxygen species are produced at low glucose and contribute to the activation of AMPK in insulin-secreting cells. *Free Radic. Biol. Med.* 52, 142–150. doi: 10.1016/j.freeradbiomed.2011.10.437
- Scott, C. B. (2008). *A Primer for the Exercise and Nutrition Sciences Thermodynamics, Bioenergetics, Metabolism*. Totowa, NJ: Springer. doi: 10.1007/978-1-60327-383-1
- Shaikh, A. Y., and Shih, J. A. (2012). Chemotherapy-induced cardiotoxicity. *Curr. Heart Fail. Rep.* 9, 117–127. doi: 10.1007/s11897-012-0083-y
- Simoneau, J. A., and Kelley, D. E. (1997). Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. *J. Appl. Physiol. (Bethesda, Md.: 1985)* 83, 166–171.
- Sin, T. K., Tam, B. T., Yu, A. P., Yip, S. P., Yung, B. Y., Chan, L. W., et al. (2015). Acute treatment of resveratrol alleviates DOX-induced myotoxicity in aged skeletal muscle through SIRT1-dependent mechanisms. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 71, 730–739. doi: 10.1093/gerona/glv175
- Smuder, A. J., Kavazis, A. N., Min, K., and Powers, S. K. (2011). Exercise protects against DOX-induced markers of autophagy signaling in skeletal muscle. *J. Appl. Physiol.* 111, 1190–1198. doi: 10.1152/jappphysiol.00429.2011
- Sparks, L. M., Xie, H., Koza, R. A., Mynatt, R., Hulver, M. W., Bray, G., et al. (2005). Required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes* 54, 1926–1933. doi: 10.2337/diabetes.54.7.1926
- Spriet, L. L. (1995). “Anaerobic metabolism during high-intensity exercise.” in *Exercise Metabolism*, ed M. Hargreaves (Champaign, IL: Human Kinetics), 1–40.
- Steinberg, G. R., Michell, B. J., van Denderen, B. J. W., Watt, M. J., Carey, A. L., Fam, B. C., et al. (2006). Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. *Cell Metab.* 4, 465–474. doi: 10.1016/j.cmet.2006.11.005
- Swain, S. M., Whaley, F. S., and Ewer, M. S. (2003). Congestive heart failure in patients treated with DOX. *Cancer* 97, 2869–2879. doi: 10.1002/cncr.11407
- Tacar, O., Sriamornsak, P., and Dass, C. R. (2013). DOX: an update on anticancer molecular action, toxicity and novel drug delivery systems. *J. Pharm. Pharm.* 65, 157–170. doi: 10.1111/j.2042-7158.2012.01567.x
- Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., et al. (2013). Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature* 496, 238–242. doi: 10.1038/nature11986
- Trostler, N., Amin, R., and Shafir, E. (1982). Increased protease activity in muscles of obese- (ob/ob) mice. *Int. J. Obes.* 6, 557–566.
- van Etten, E. W., ten Kate, M. T., Snijders, S. V., and Bakker-Woudenberg, I. A. (1998). Administration of liposomal agents and blood clearance capacity of the mononuclear phagocyte system. *Antimicrob. Agents Chemother.* 42, 1677–1681.
- van Norren, K., van Helvoort, A., Argilés, J. M., van Tuijl, S., Arts, K., Gorselink, M., et al. (2009). Direct effects of DOX on skeletal muscle contribute to fatigue. *Br. J. Cancer* 100, 311–314. doi: 10.1038/sj.bjc.6604858
- Viollet, B., Lantier, L., Devin-Leclerc, J., Hébrard, S., Amouyal, C., Mounier, R., et al. (2009). Targeting the AMPK pathway for the treatment of Type 2 diabetes. *Front. Biosci. (Landmark Ed.)* 14, 3380–3400. doi: 10.2741/3460
- Wang, B., Yang, G., Liang, X., Zhu, M., and Du, M. (2014). Grape seed extract prevents skeletal muscle wasting in interleukin 10 knockout mice. *BMC Complement. Altern. Med.* 14:162. doi: 10.1186/1472-6882-14-162
- Wang, C., Li, J., Liu, Q., Yang, R., Zhang, J. H., Cao, Y. P., et al. (2011). Hydrogen-rich saline reduces oxidative stress and inflammation by inhibit of JNK and NF- κ B activation in a rat model of amyloid-beta-induced Alzheimer's disease. *Neurosci. Lett.* 491, 127–132. doi: 10.1016/j.neulet.2011.01.022
- Wapstra, F. H., van Goor, H., de Jong, P. E., Navis, G., and de Zeeuw, D. (1999). Dose of DOX determines severity of renal damage and responsiveness to ACE-inhibition in experimental nephrosis. *J. Pharmacol. Toxicol. Methods* 41, 69–73. doi: 10.1016/S1056-8719(99)00015-5
- Yu, A. P., Pei, X. M., Sin, T. K., Yip, S. P., Yung, B. Y., Chan, L. W., et al. (2014). Acylated and unacylated ghrelin inhibit DOX-induced apoptosis in skeletal muscle. *Acta Physiol.* 211, 201–213. doi: 10.1111/apha.12263
- Zuo, L., Otenbaker, N. P., Rose, B. A., and Salisbury, K. S. (2013a). Molecular mechanisms of reactive oxygen species-related pulmonary inflammation and asthma. *Mol. Immunol.* 56, 57–63. doi: 10.1016/j.molimm.2013.04.002
- Zuo, L., Roberts, W. J., Tolomello, R. C., and Goins, A. T. (2013b). Ischemic and hypoxic preconditioning protect cardiac muscles via intracellular ROS signaling. *Front. Biol.* 8:305–311. doi: 10.1007/s11515-012-1225-z
- Zuo, L., Zhou, T., Pannell, B. K., Ziegler, A. C., and Best, T. M. (2015). Biological and physiological role of reactive oxygen species - the good, the bad and the ugly. *Acta Physiol.* 214, 329–348. doi: 10.1111/apha.12515

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Supriya, Tam, Pei, Lai, Chan, Yung and Siu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.