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Effect of n-3 Polyunsaturated Fatty Acids on Induction of Hypoxia Inducible Factor-2 Alpha in C_2C_{12} Myotubes

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Abstract: Results from different trails have provided evidence of protective effects of n-3 Polyunsaturated Fatty Acids (n-3 PUFAs) on heart and skeletal muscle. But the induction of Hypoxia Inducible Factors (HIFs) by n-3 PUFAs in these protective effects has never been reported before. The aim of this study was to find out if the marine n-3 fatty acids Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) and of the plant-derived Alpha Linolenic Acid (ALA) and Stearidonic Acid (SDA) can induce HIFs in C_2C_{12} myotubes and subsequent effect on cell metabolism. The n-3 PUFAs, especially DHA, EPA and SDA increased the levels of protein and mRNA in HIF-2α at 72 h. Meanwhile, n-3 PUFAs also elevated the expression of HIF related transcriptional factors like PDK4 and PPARα at 72 h. The reprogramming of basal metabolism in C_2C_{12} myotubes increased in *GLUT4* and *CPT1b* gene expression by treatment of n-3 PUFAs at 72 h. UCP2 was increased by n-3 PUFAs for attenuating production of ROS at 72 h. n-3 PUFAs, especially DHA, EPA and SDA could induce HIF-2α which is associated with upregulation of PDK4 by activation of PPARα. This process also implies a reprogramming of basal metabolism and oxidative damage protection in C_2C_{12} myotubes.

Key words: n-3 PUFAs, HIF-2α, PDK4, PPARα, basal metabolism, skeletal muscle

INTRODUCTION

Heart diseases like Coronary Heart Disease (CHD), Myocardial Infarction (MI) and myocardial ischemia are causing more and more deaths in recent decades all over the world because of the intake of high proportion energy and unbalanced dietary habits (Jalali-Khanabadi *et al*., 2006; Shekhar *et al*., 2006; Mazloom *et al*., 2008; Xu *et al*., 2010). So, a lot of research has been done for preventing these diseases by using variable chemicals (Granzotto *et al*., 2005; Jun *et al*., 2007; Anand *et al*., 2008; Ara *et al*., 2008; Nishimura *et al*., 2011; Liu *et al*., 2011).

Among these different chemicals, special attention has been paid to n-3 Polyunsaturated Fatty Acids (n-3 PUFAs) (Fang *et al*., 2011; Joensen *et al*., 2010). The n-3 PUFAs refer to a family of unsaturated fatty acids which mainly originate from the plant-derived n-3 fatty acids: Alpha Linolenic Acid (ALA; C18:3 n-3) and Stearidonic Acid (SDA; C18:4 n-3) or the marine n-3 fatty acids: Docosahexaenoic Acid (DHA; C22:6 n-3) and Eicosapentaenoic Acid (EPA; C20:5 n-3) (Mu, 2008; Ruiz-Lopez *et al*., 2009; Lorgeril de and Salen, 2004).

The mechanism of beneficial effects of n-3 PUFAs on cardiovascular diseases is mediated through different ways. In clinical experiments, the effect of n-3 PUFAs in patients is predominantly on antiatherogenic in lipid metabolism (Kris-Etherton *et al*., 2002; Vannice and Kelly, 2004; De Lorgeril *et al*., 1994). In animal models, besides changing lipid parameters in blood serum, dietary n-3 PUFAs supplementation can protect against myocardial resistance to ischemia-reperfusion injury (Ogita *et al*., 2003), reduce myocardial infarct sizes and arrhythmias (Xiao *et al*., 2008) or decrease O_2 consumption and increase oxygen utilization efficiency (Pepe and McLennan, 2002).

The main explanations of n-3 PUFAs for reducing cardiac oxygen consumption and increasing the efficiency of cardiac metabolism are concentrated on the incorporation of n-3 PUFAs into myocardial membrane (Pepe and McLennan, 2002) and feeding related cellular calcium regulation (Singh *et al*., 2010). However, it was recently noted that cardiomyocytes can activate an endogenous oxygen-sensitive transcriptional programme when the myocardium is challenged by a low oxygen supply in areas of acute or chronic ischaemia. This process is mediated by Prolyl Hydroxylases (PHDs) which act as oxygen sensors to control the expression of Hypoxia-Inducible Factors (HIFs) (Bernhardt *et al*., 2007). PHDs, present in three forms in mammalian cells are 2-oxoglutarate dioxygenases designated PHD1-PHD3

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(Epstein *et al*., 2001). HIF-α proteins are maintained at low steady-state level under normoxic condition via hydroxylation by HIF Prolyl Hydroxylases (PHDs) (Jaakkola *et al*., 2001). HIF consists of an unstable α subunit and a stable β subunit that binds DNA at specific locations termed Hypoxia Response Elements (HERs) to regulate many genes expressions related to hypoxia (Wiesener *et al*., 1998). HIF-α subunit is regulatory and unique to the hypoxic response. HIF-α subunit is constitutive and involved in xenobiotic response. Three different genes encoding HIF-α subunit are found in mammals: HIF-1α to HIF-3α (Maxwell, 2005). Among these three HIF-α isoforms, HIF-2α in particular shows a unique ability to induce metabolic reprogramming which ultimately makes mitochondrion harmless but less active in certain conditions by regulating expression of numerous genes (Aragones *et al*., 2008).

Because n-3 PUFAs showed protective effects on skeletal muscle that shared similar mechanism as myocardium (Peoples and McLennan, 2010; Ayre and Hulebrt, 1996) and a beneficial effect on ischemia and Ischemia/Reperfusion (I/R) injury of tissues by normoxic induction of HIF-2α (Aragones *et al*., 2008), we hypothesize that n-3 PUFAs may have an inhibitory effect on PHD1 which is contributed to HIF stabilization and protection of skeletal muscle. We employed C_2C_{12} murine skeletal myoblasts as a model to determine if these effects are associated with role of prolyl hydroxylase inhibitor and induction of HIF-2α. We used Linoleic Acid (LA; C18:2 n-6)-one of the important n-6 fatty acids as a positive control to compare with the effect of n-3 PUFAs.

MATERIALS AND METHODS

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Cellgro (Manassas, VA). Penicillin/streptomycin, Phosphate-Buffered Saline (PBS), fatty acid-free Bovine Serum Albumin (BSA) and trypsin-EDTA were purchased from Sigma Chemical (St. Louis, MO). LA, ALA, DHA, EPA and SDA (all FAs were with purity >99%) were obtained from Nu-Chek (Elysan, MN). Murine skeletal muscle-derived C_2C_{12} myoblasts were purchased from American Type Culture Collection (Manassas, VA), No. CRL-1772. Antibodies for PHD1and GAPDH were purchased from Bethyl (Montgomery, TX). Antibodies for HIF-2α, PPARα and secondary antibody coupled to Horse Radish Peroxidase (HRP) were purchased from Abcam (Cambridge, MA).

Cell culture: Mouse skeletal muscle cell line, C_2C_{12} myoblasts were maintained in DMEM containing 4.5 g L^{-1} glucose supplemented with 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin at 37°C under a 5% CO_2 atmosphere in 100 mm culture dishes or six-well plates. About 2 days after the cells reached confluence, differentiation was induced by switching the growth medium to DMEM supplemented with 2% horse serum (differentiation medium). The medium was changed every other day. To examine the effect of n-3 PUFAs, after 4 days differentiation, C_2C_{12} myotubes were treated with 50 μM BSA, LA, ALA, DHA, EPA and SDA for 72 h. LA (14.0 mg), ALA (13.9 mg), DHA (16.4 mg), EPA (15.1 mg) and SDA (13.8 mg) were dissolved in 1.6 mL of 0.1 M KOH (50°C for 10 min) and incubated with 40 mL of 1.25 mM fatty acid free bovine serum albumin solution overnight at 4°C. PBS 1X was added until the volume of solution reached to 50 mL. Finally, fatty Acid-bovine Serum Albumin (BSA) complex was filter-sterilized using 0.22 mm filter (Millipore, Billerica, MA) to make 1mM stock solution (pH 7.2). BSA solution without fatty acid was used as a vehicle control. LA was used as a positive control.

Western blot analysis: C_2C_{12} myotubes were washed with PBS and cell lysates were isolated in RIPA buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% Sodium Deoxycholate and 0.1% SDS) supplemented with protease inhibitors (1 mM PMSF, 5 mg mL^{-1} aprotinin and 5 mg mL^{-1} leupeptin) and phosphatase inhibitors (1 mM Na_3VO_4 and 1 mM NaF) and centrifuged at 12,000 r.p.m. for 20 min at 4°C. Protein concentration was determined by Bio-Rad protein assay (Hercules, CA) using 1.4 mg mL^{-1} BSA as the standard. Proteins (25 μg) were separated on SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, MA). After blocking with a specific primary antiserum in Tris Buffered Saline (TBS) containing 0.05% Tween-20 (TBS-T) and 5% non-fat dry milk at 4°C overnight, the membrane was incubated with each antibody (anti-PHD1, anti-HIF-2α, anti-PPARα, anti-GAPDH) at 4°C overnight. Finally, after three washes with TBS-T, the blots were incubated with secondary antibody coupled to HRP for 1 h at room temperature, visualized using ECL Plus Western Blotting Detection System (GE Healthcare, Buckin-Ghamshire, UK) and quantified by Kodak Image Station Software (Scion, Frederick, MD).

RNA isolation and one-step reverse transcriptase, real-time-polymerase chain reaction: After treatment, total RNA from C_2C_{12} myotubes was isolated with Trizol Reagent (Invitrogen, Carlsbad, CA) according to the

Table 1: Information of gene primers used for RT-PCR

Gene	Assay ID	Ref_Seq	Exon boundary	Assay location
Arnt2	Mm00476004_m1	-	12-13	1343
Cpt1b	Mm00487200_m1	NM_009948.2	17-18	2228
EGLN2	Mm00519067_m1	NM_053208.4	2-3	1227
EPAS1	Mm01236112_m1	NM_010137.3	6-7	1192
FAS	Mm00662291_g1	NM_007988.3	14-15	2826
GLUT4	Mm00436615_m1	NM_009204.2	9-10	1328
HIF-1α	Mm00468869_m1	NM_010431.2	4-5	879
HIF-3α	Mm00469373_m1	-	10-11	1407
PDK4	Mm01166879_m1	NM_013743.2	3-4	454
PPARα	Mm00440939_m1	NM_011144.6	7-8	1371
UCP2	Mm00627597_m1	NM_011671.4	1-2	119

manufacturer's instructions. A one-step was performed in a Veriti® 96 well Fast Thermal Cycler (Applied Biosystems, Foster City, CA) to reverse transcribe the mRNA into cDNA which was then amplified using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) and TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA). All reactions were performed in 96 well plates with a final volume of 20 μL per well. Cycling conditions were 2 min at 50°C followed by 10 min at 95°C to activate the HotStarTaq DNA Polymerase, 40 cycles of 15 sec at 94°C and 1 min at 60°C. TaqMan gene expression assays were obtained from Applied Biosystems (Foster city, CA). The information of gene primers used for amplification is shown in Table 1. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was amplified and used as a within-sample normalizing control (Assay ID Mm99999915_g1). Data was obtained by the method referred to in former research (Platt and El-Sohemy, 2009).

Statistical analyses: Results were expressed as mean±SE with at least triplication in each group. All differences were analyzed using a one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test. $p<0.05$ was considered significant. All data was analyzed using SAS Software (Version 9.2, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Gene expression of HIFs and PHD1: The effect of n-3 PUFAs on gene expression of three types of HIF-α subunits, HIF-β subunit and HIF-α subunit hydroxylase was determined. There was no significant difference among different gene expression at treatment of 24 h. As shown in Fig. 1a, ALA, EPA and SDA had effect on *HIF-1α* gene expression at 72 h. As shown in Fig. 1b, >2 fold increase in EPAS1 (*HIF-2α*) gene expression was noted in C_2C_{12} myotubes in the presence of DHA, EPA and SDA at 72 h ($p<0.05$). *HIF-3α* gene expression was unaltered by all treatments at different time (Fig. 1c). The expression of ARNT2 (*HIF-α*) gene was significantly elevated by both EPA and SDA relative to CTRL and LA at 72 h ($p<0.05$) (Fig. 1d). Interestingly, DHA, EPA and SDA also increased the expression of EGLN2 (*PHD1*) gene, compared with CTRL and LA both at 48 and 72 h ($p<0.05$) (Fig. 1e).

Protein expression of HIF-2α and PHD1: After determining the effect of n-3 PUFAs on HIFs and PHD1 in mRNA level, protein expression of HIF-2α and PHD1 by C_2C_{12} myotubes was also determined. As shown in Fig. 2, no difference of PHD1 protein was observed in all groups at different time. The effect of DHA, EPA and SDA on increasing HIF-2α protein expression was shown at 72 h in Fig. 2c.

Gene and protein expression of PPARα pathway: As recently noted (Aragones *et al*., 2008) enhanced expressions of PDK4 and PPARα associated with stabilized HIF-2α were shown in mouse muscle. To determine if the effect of n-3 PUFAs on stabilization of HIF-2α might be mediated by PPARα pathway, the effects of n-3 PUFAs on PDK4 and PPARα expression in C_2C_{12} myotubes were measured. There was no significant difference in both PDK4 and *PPARα* gene expression at treatment of 24 h. Compared with CTRL and LA ($p<0.05$), a significant elevation of PDK4 gene expression was evident in C_2C_{12} myotube treated with DHA, EPA and SDA at 72 h as shown in Fig. 3a. The higher *PPARα* gene expression was observed with SDA treatment ($p<0.05$) but no difference was observed in other groups relative to CTRL and LA at 72 h (Fig. 3b). Unlike the *PPARα* gene expression, DHA, EPA and SDA increased PPARα protein expression in comparison with CTRL and LA at 72 h (Fig. 3e).

Gene expression of glucose and lipid metabolism enzymes: There was no significant difference among *GLUT4*, *CPT1b* and *UCP2* gene expression at treatment of 24 h. As a marker for glucose metabolism, *GLUT4* gene expression was only increased by SDA compared with CTRL and LA at 72 h ($p<0.05$) (Fig. 4a). For a key enzyme of fatty acid β-oxidation, *CPT1b* gene expression was elevated by over 3 fold in the treatment of EPA and SDA at 72 h ($p<0.05$) (Fig. 4b). However, gene expression of FAS was undetermined in all groups at different time. DHA, EPA and SDA significantly increased *UCP2* gene

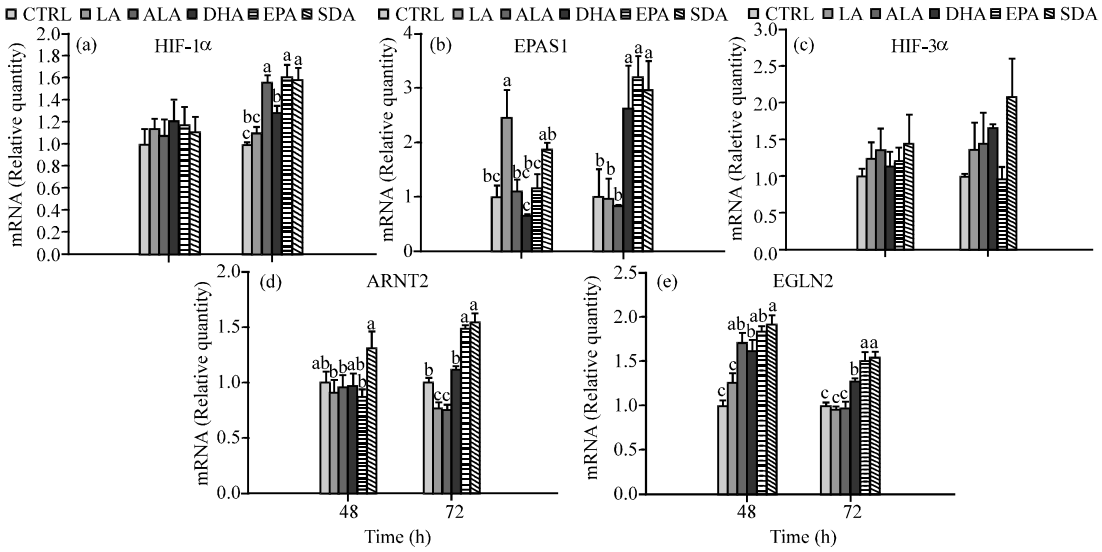

Fig. 1: Effects of individual n-3 PUFAs on gene expression of HIFs and PHD1 in C_2C_{12} myotubes. Cells were cultured in 6 well plates and treated for 24, 48 and 72 h in medium with BSA, LA, ALA, DHA, EPA and SDA at concentration of 50 μM. Values are expressed as mean±SE (n = 4); a) The effect of individual n-3 PUFAs on HIF-1α; b) EPAS1 (HIF-2α); c) HIF-3α; d) ARNT2 (HIF-β) and e) EGLN2 (*PHD1*) gene expression was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Values with different superscript letters are significantly different at $p<0.05$

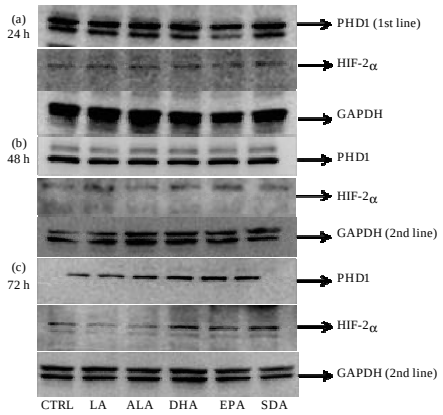

Fig. 2: Effects of individual n-3 PUFAs on protein expression of PHD1 and HIF-2α at a) 24 h, b) 48 h and c) 72 h in C_2C_{12} myotubes. Cells were cultured in 100 mm dishes and treated for 24, 48 and 72 h in medium with BSA, LA, ALA, DHA, EPA and SDA at concentration of 50 μM. GAPDH is used as internal control for western blotting analysis

expression ($p<0.05$) relative to CTRL and LA at 72 h (Fig. 4c). In this study, n-3 PUFAs did not show the ability to inhibit PHD1 expression. But interestingly, n-3 PUFAs especially DHA, EPA and SDA, played a role in stabilizing HIF-2α in the normoxia environment and affected expression of HIF related transcriptional genes without inhibition of PHD1 at 72 h. This is a new discovery of well-studied n-3 PUFAs on skeletal muscle protective effect. It is suggesting that n-3 PUFAs may induce HIF-2α in a PHD1 independent way.

Specifically, n-3 PUFAs species increased HIF-α expression like HIF-1α and HIF-2α in mRNA or protein level. The protective effect of HIFs within different tissues has already been noted before (Aragones *et al*., 2008; Hyvarinen *et al*., 2010; Natarajan *et al*., 2006). However, there are some debates about the expression of HIF-α in mRNA and in protein level and also about the constitutive expression of HIF-α. Research indicated that there was no significant increase in the mRNA expression of EPAS1 (Forsyth *et al*., 2008; Westfall *et al*., 2008). This may be due to posttranscriptional regulation as found in specific cell types (Huang *et al*., 1998; Lang *et al*., 2002). In general, HIF-α is constitutively expressed and heterodimerizes with HIF-α subunit in the nucleus to form a complex which binds to hypoxia-responsive elements in enhancers and promoters of oxygen-responsive genes under hypoxic conditions (Westfall *et al*., 2008). In contrast to this however, former research (Lang *et al*., 2002) showed that HIF-α mRNA expression was upregulated under hypoxia. Consistent with this study, the expression of ARNT2 was increased by supplementation of n-3 PUFAs, especially EPA and SDA which may reflect differences in both the methodology and cell lines used.

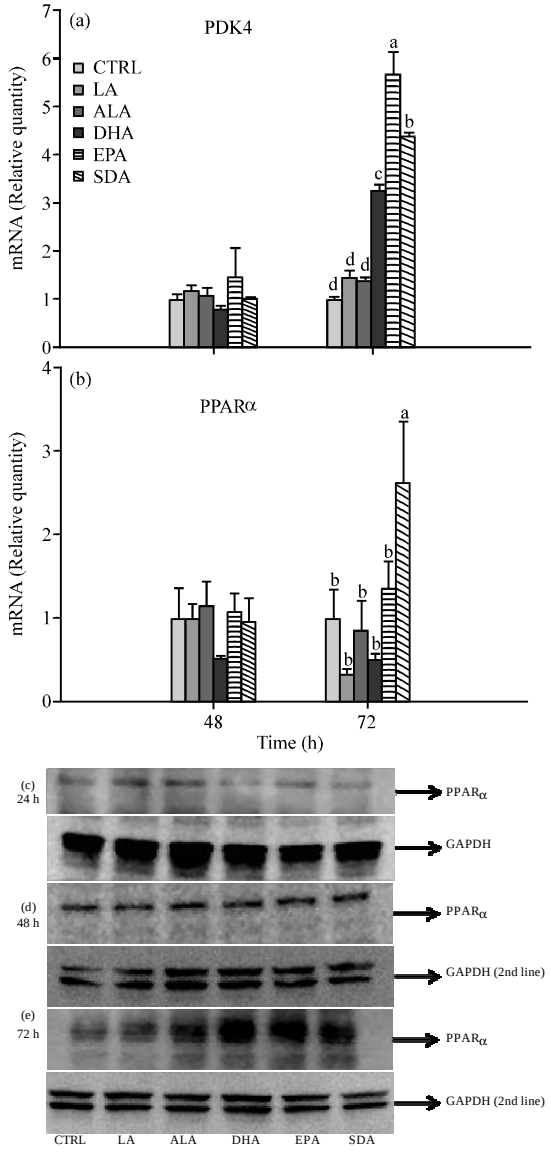

Fig. 3: Effects of individual n-3 PUFAs on gene and protein expression of PPARα pathway in C_2C_{12} myotubes. Cells were cultured in six-well plates or 100 mm dishes and treated for 24, 48 and 72 h in medium with BSA, LA, ALA, DHA, EPA and SDA at concentration of 50 μM. Values are expressed as mean±SE (n = 4). a) The effect of individual n-3 PUFAs on PDK4; b) *PPARα* gene expression was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Values with different superscript letters are significantly different at $p<0.05$; c) The effect of individual n-3 PUFAs on PPARα protein expression at different time is shown 24 h, d) 48 and e) 72 h. GAPDH is used as internal control for western blotting analysis

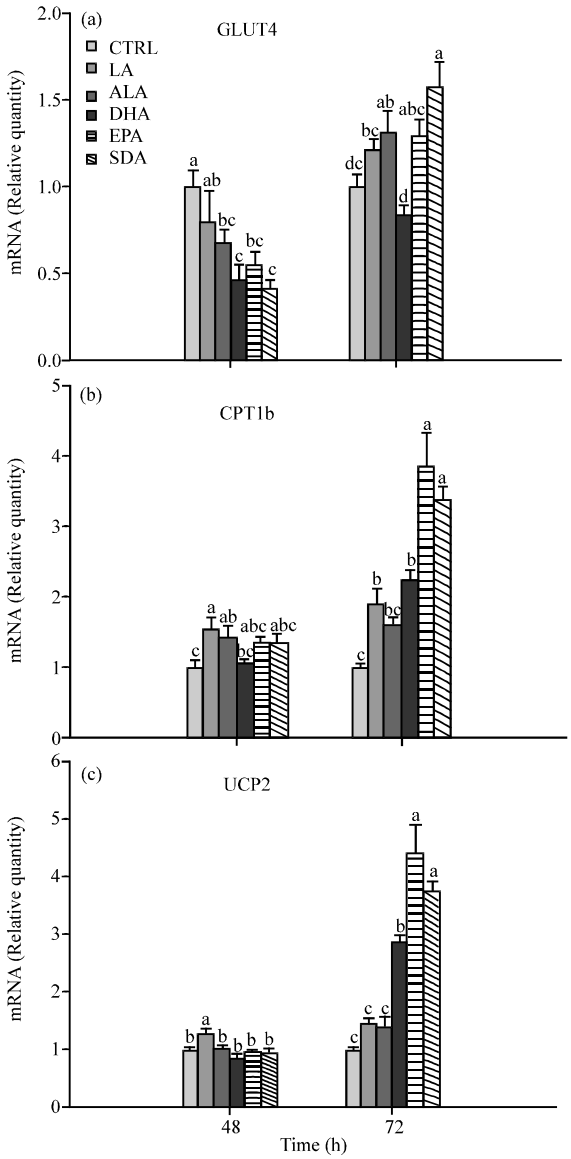

Fig. 4: Effects of individual n-3 PUFAs on gene expression of glucose and lipid metabolism enzymes in C_2C_{12} myotubes. Cells were cultured in six-well plates and treated for 24, 48 and 72 h in medium with BSA, LA, ALA, DHA, EPA and SDA at concentration of 50 μM. Values are expressed as mean±SE (n = 4), a) The effect of individual n-3 PUFAs on *GLUT4*, b) *CPT1b* and c) *UCP2* gene expression was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Values with different superscript letters are significantly different at $p<0.05$

The possible mechanism inducing protective effect of HIFs, especially HIF-2α, is linked to an increase in the

expression of PDK4 (Kelly, 2008). In the normoxic environment, pyruvate enters the Tricarboxylic Acid (TCA) Cycle inside the mitochondrion where it generates ATP in the presence of oxygen. But in the hypoxia environment like heart ischemia, entry of pyruvate is restricted by expression of PDK4 which is associated with induction of HIF-2α (Aragones *et al*., 2008; Wu *et al*., 2001). In this study, *PDK4* gene expression was upregulated in myotubes treated DHA, EPA and SDA.

PPARα is known to activate the *PDK4* gene (Wu *et al*., 2001). Research on the hibernating mammal model also showed the level of PDK4 mRNA increased greatly by activation of PPARα during hibernation (Buck *et al*., 2002). Hearts from PPARα agonist clofibrate-treated rats had an improved recovery of post-ischemic contractile function and reduced Ischemia/Reperfusion (I/R)-induced infarct size. In hearts of rats the content of the principal n-3 PUFA (DHA) was markedly increased while the principal n-6 PUFA (LA) and n-6/n-3 ratio was significantly reduced (Tian *et al*., 2006). In this study, we found that DHA, EPA and SDA significantly increased PPARα protein expression. This result, combined with *PDK4* gene expression and HIF-2α expression, suggests that PPARα activated by n-3 PUFAs with induction of HIF-2α can initiate *PDK4* gene expression and this process implies a reprogramming of basal metabolism in cells, especially in energy production and utilization like glucose metabolism and fatty acid metabolism. Glycolytic flux was increased in mouse muscle fiber in low oxygen conditions (Argarones *et al*., 2008). As a maker for glucose metabolism, SDA increased *GLUT4* gene expression significantly in this study. Combined, n-3 PUFAs may induce more glucose entering cell cytoplasm to generate ATP by glycolysis when HIF-2α was induced. CPT1b is known as a rate-limiting enzyme in fatty acid α-oxidation which transfers fatty acids through mitochondrial membrane to be oxidized within the mitochondrial matrix (McGarry and Brown, 1997). *In vivo* experiment, rats fed with fish oil had a significantly greater skeletal muscle CPT1 specific activity (Power and Newsholme, 1997). Consistent with this study, both EPA and SDA significantly elevated *CPT1b* gene expression. In another *in vivo* experiment, dietary fish oil reduced skeletal muscle oxygen consumption in hindlimbs of rats (Peoples and McLennan, 2010). Along with this study, fatty acids became the primary source of fuel in hibernating mammals, who consumed less oxygen for energy production because of the inhibition of carbohydrate oxidation by the enzyme PDK4 (Buck *et al*., 2002). Former research and this study suggest that n-3 PUFAs could switch the energy source from carbohydrate to fatty acid by the activation of *PDK4* gene in PPARα pathway to enable C_2C_{12} myotubes to consume oxygen more efficiently.

As an important energy expenditure parameter, the effect of Uncoupling Proteins (UCPs) has become prominent in the field of thermogenesis, especially UCP1 (Brand and Esteves, 2005). But for UCP2 and UCP3, there is a consensus that the primary function of UCP2 and UCP3 is to attenuate mitochondrial production of free radical to protect against oxidative damage, degenerative disease and aging rather than to promote gross thermogenesis or energetic inefficiency (Brand *et al*., 2002; Casteilla *et al*., 2001). Oxidative damage caused by Reactive Oxygen Species (ROS) is produced in mitochondrion which can trigger the toxic effects living with oxygen. In contrast, oxygen depletion (hypoxia) also increases mitochondrial ROS that is detrimental to cells unless attenuated (Guzy and Schumacker, 2006). In this study, the *UCP2* gene expression in DHA, EPA and SDA treatment was elevated greatly which is consistent with former research done on effect of elucidating UCP2 on attenuating ROS production (Arsenijevic *et al*., 2000; Cortez-Pinto *et al*., 2001).

CONCLUSION

To the knowledge this study is the first to examine the effect of n-3 PUFAs on induction of HIF-2α in C_2C_{12} myotubes. Interestingly, this induction is not associated with inhibition of PHD1 which could be associated with other PHDs. Several lines of evidence suggest the protective effect of n-3 PUFAs on induction of HIF-2α plays a part by upregulation of *PDK4* gene expression which is activated by PPARα. This process can imply a reprogramming of basal metabolism in C_2C_{12} myotubes to consume oxygen more efficiently by switching carbohydrate oxidation to glycolysis and fatty acid oxidation. Meanwhile, n-3 PUFAs also increased *UCP2* gene expression to attenuate the damage of ROS. This study provides a new interpretation of protective effect in skeletal muscle and myocardium by n-3 PUFAs, especially DHA, EPA and SDA.

NOMENCLATURE

ALA = Alpha Linolenic Acid
ARNT2 = Aryl hydrocarbon Receptor Nuclear Translocator 2
CPT1b = Carnitine Palmitoyltransferase 1b
DHA = Docosahexaenoic Acid
EGLN2 = Egl Nine homolog 2
EPA = Eicosapentaenoic Acid

EPAS1 = Endothelial PAS domain-containing protein 1
FA = Fatty Acid
GAPDH = Glyceraldehyde 3-Phosphate Dehyd-rogenase
GLUT4 = Glucose Transporter Type 4
HIF = Hypoxia Inducible Factor
LA = Linoleic Acid
PDK4 = Pyruvate Dehydrogenase Kinase 4
PPARα = Peroxisome Proliferator-Activated Receptor Alpha
PUFAs = Polyunsaturated Fatty Acids
SDA = Stearidonic Acid
UCP2 = Mitochondrial Uncoupling Protein 2

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