Effects of Eicosapentaenoic Acid and Docosahexaenoic Acid on Mitochondrial DNA Replication and PGC-1α Gene Expression in C2C12 Muscle Cells

Mak-Soon Lee, Yoonjin Shin, Sohee Moon, Seunghae Kim, and Yangha Kim

Department of Nutritional Science and Food Management, College of Science and Industry Convergence, Ewha Womans University, Seoul 03760, Korea

ABSTRACT: Mitochondrial biogenesis is a complex process requiring coordinated expression of nuclear and mitochondrial genomes. The peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) is a key regulator of mitochondrial biogenesis, and it controls mitochondrial DNA (mtDNA) replication within diverse tissues, including muscle tissue. The aim of this study was to investigate the effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on mtDNA copy number and PGC-1α promoter activity in C2C12 muscle cells. mtDNA copy number and mRNA levels of genes related to mitochondrial biogenesis such as PGC-1α, nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (Tfam) were assayed by quantitative real-time PCR. The PGC-1α promoter from −970 to +412 bp was subcloned into the pGL3-basic vector, which includes a luciferase reporter gene. Both EPA and DHA significantly increased mtDNA copy number, dose and time dependently, and up-regulated mRNA levels of PGC-1α, NRF1, and Tfam. Furthermore, EPA and DHA stimulated PGC-1α promoter activity in a dose-dependent manner. These results suggest that EPA and DHA may modulate mitochondrial biogenesis, which was partially associated with increased mtDNA replication and PGC-1α gene expression in C2C12 muscle cells.

Keywords: EPA, DHA, mtDNA, PGC-1α, muscle cells

INTRODUCTION

Skeletal muscle plays a vital role in whole body energy balance as a major site of mitochondrial oxidative metabolism. To promote mitochondrial number and function in skeletal muscle, it is important to activate specific signal transduction mechanisms that stimulate peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α). Overexpressing PGC-1α in skeletal muscle of mice was reported to increase the amount of mitochondria (1). PGC-1α is a primary regulator of mitochondrial biogenesis by virtue of its ability to co-activate and augment the expression and activity of several transcription factors (2,3). Activated PGC-1α increases expression of nuclear respiratory factors 1 (NRF1) and mitochondrial transcription factor A (Tfam). NRF1 subsequently up-regulates Tfam to stimulate mitochondrial DNA (mtDNA) transcription and replication (4-6).

Omega-3 fatty acids are mainly found in the foods, such as fish oil, some plants and nut oils (7). Dietary omega-3 polyunsaturated fatty acids (ω-3 PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential nutrients for human health. In humans, EPA and DHA promote improved insulin sensitivity (8), lipid oxidation (9), and reduce body weight (10,11). The ω-3 PUFA have been shown to increase the expression of genes involved in mitochondrial biogenesis in white fat of C57BL/6J mice (12). Furthermore, a previous study showed that EPA induced mitochondrial biogenesis as consequence of transcriptional activity of PGC-1α and Tfam in C6 glioma cells (13). However, whether EPA and DHA directly regulate the mtDNA replication and PGC-1α activation in skeletal muscle cells in vitro remains unresolved.

Therefore, we investigated the direct effects of EPA and DHA on the expression of genes involved in mitochondrial biogenesis, mtDNA copy number, and PGC-1α promoter activity in C2C12 muscle cells.
MATERIALS AND METHODS

Materials and reagents
EPA, DHA, palmitate (PA), butylated hydroxytoluene (BHT), α-tocopherol, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The C2C12 mouse muscle cell line was obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco’s modified Eagle’s medium (DMEM), glutamine, penicillin-streptomycin, fetal bovine serum (FBS), and TRIzol reagent were obtained from Invitrogen (Carlsbad, CA, USA). Moloney murine leukemia virus (M-MLV) reverse transcriptase, pGEM easy vector, pGL3 basic vector, and luciferase reporter assay system (Promega, Madison, WI, USA). pCMV-β galactosidase was obtained from Clontech Laboratories, Inc. (Palo Alto, CA, USA). Puregene DNA isolation kit were purchased from Qiagen (Chatsworth, CA, USA). Mlu I and Xho I were obtained from Takara (Tokyo, Japan).

Preparation of EPA, DHA, and PA
Non-esterified EPA (C20:5, ω-3), DHA (C22:6, ω-3), and PA (C16:0) were dissolved in 95% ethanol. EPA, DHA, and PA were combined with 7.5% BSA by stirring for 1 h at 37°C. Fatty acid/BSA complexes were added 0.1% BHT and 20 μM α-tocopherol to minimize oxidation. The saturated fatty acid, PA, was used to compare the relative change to unsaturated fatty acids (EPA and DHA).

Cell culture
Mouse C2C12 myoblasts were cultured in DMEM supplemented with 10% FBS and 1 U penicillin-streptomycin at 37°C in a 5% CO2 atmosphere. When C2C12 cells reached 90% confluency, differentiation was induced by incubation for 5 days with differentiation medium containing 2% horse serum. For the gene expression assay at mRNA level, differentiated C2C12 muscle cells were treated without (control) or with 50 μM PA, EPA, or DHA in serum-free medium for 24 h. For promoter activity assay, cells were cultured in serum-free media for 40 h with 0 (control), 1, 10, or 50 μM EPA and DHA. Control cells were treated with 0.1% BHT and 20 μM α-tocopherol without fatty acids. All measurements were performed in triplicate.

mtDNA copy number
The genomic DNA was extracted from muscle with a Puregene DNA isolation kit according to the manufacturer’s instructions. mtDNA copy number was calculated by real-time quantitative polymerase chain reaction (PCR) by measuring a mitochondrial gene [cytochrome oxidase subunit 1 (Cox1) vs. a nuclear gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH)].

Real-time quantitative PCR
Total RNA was extracted from cells with TRIzol reagent. The corresponding cDNA was synthesized from 4 μg of RNA with M-MLV reverse transcriptase. After cDNA synthesis, real-time quantitative PCR was performed by using Universal SYBR Green PCR Master Mix on a fluorometric thermal cycler (Corbett Research, Mortlake, Australia). Primers were designed by the program Primer3 (14). Sequences of the sense and antisense primers are shown in Table 1. The ΔΔCt method was used for relative quantification. The ΔΔCt value for each sample was determined by calculating the difference between the Ct value of the target gene and the Ct value of β-actin as a reference gene. The normalized expression level of each target gene was calculated as 2−ΔΔCt. Values are expressed as fold of the control.

Construction of PGC-1α reporter gene
The human PGC-1α gene promoter was generated by PCR of human genomic DNA, using primers recognizing exon 1 of the PGC-1α gene (970 bp upstream to 412 bp downstream). The sequence information was deposited with GenBank (Accession no. AF108193). The primers of the PGC-1α gene promoter were designed such that the amplified promoter fragment was flanked by two restriction sites (Mlu I and Xho I). The 5’-primer, bearing a Mlu I site, was 5’TGT ACG CGT CCT TCA GTT CAC AGA CAT ‘TCT-3’, and the 3’-primer, bearing a Xho I site, was 5’TCT CTC GAG ACA GTG CCA AAG TCA AGA CAT TCT-3’. The amplified promoter fragment was subcloned into the pGEM-T easy vector. It was then inserted into the pGL3 basic vector, which includes a luciferase reporter gene.

Table 1. Primers used for quantitative real-time polymerase chain reaction (PCR)

| Gene   | GeneBank No. | Primer sequence (5’-3’)          |
|--------|--------------|----------------------------------|
| β-actin| NM_007393    | Forward GAGACCTGACAGACTACCTCA    |
|        |              | Reverse GTGTTCAATAGTGATGACCT     |
| NRF1   | NM_010938    | Forward AAGTATTCCACAGGGTGGGG     |
|        |              | Reverse TGGTGCGCTGAGTTTGTTT      |
| PGC-1α | NM_008904    | Forward GGGCCAAAACAGAGAGAGAG    |
|        |              | Reverse GTTTGCCTGACCTGCTA       |
| Tfam   | NM_009360    | Forward GAGGCCAGTGTGAACCAGTG    |
|        |              | Reverse GTAGTGGCTGCTGCCCTGA     |

NRF1, nuclear respiratory factor 1; PGC-1α, peroxisome proliferative activated receptor gamma coactivator 1 alpha; Tfam, mitochondrial transcription factor A.
Transfection and luciferase assay
Transfection experiments were carried out with Super-
fect reagent according to the manufacturer’s instruc-
tions. The plasmids used were 2 μg of PGC-1α/luc re-
porter gene and 1 μg of pCMV-β-galactosidase as an in-
ternal standard to adjust for transfection efficiency. The pG
L3-basic vector was used as a vector control. Three
hours after transfection, cells were treated with 0 (con-
trol), 1, 10, or 50 μM EPA or DHA in 1% BSA serum-free
medium for 40 h.

For the luciferase assay, cells were washed with PBS
and harvested with lysis buffer. PGC-1α promoter ac-
vity was measured with the luciferase reporter assay sys-
tem and a TD 20/20 luminometer (Turner Designs, Sun-
yvale, CA, USA). β-Galactosidase activity was assayed
enzymatically by using o-nitrophenyl-β-D-galactopyra-
noside as a substrate. Luciferase activity was calculated in
relative light units and normalized to β-galactosidase ac-
vity.

Statistical analysis
Data are expressed as mean±standard deviation (SD).
Statistical analyses were performed using SPSS software
version 19 (IBM Corporation, Armonk, NY, USA). Sig-
nificant differences among the treatment groups were
assessed by one-way analysis of variance (ANOVA) and
Tukey’s multiple comparison tests. P<0.05 was consid-
ered to indicate a statistically significant difference.

RESULTS
Effects of EPA and DHA on mtDNA copy number
To investigate the effect of EPA and DHA on mtDNA
copy number, we determined the ratio of mtDNA to nu-
clear DNA (nDNA). Differentiated C2C12 muscle cells
were incubated in the presence of fatty acids with differ-
ent degrees of saturation, including 50 μM PA (satu-
rated) and various concentrations (0, 1, 10, or 50 μM) of
EPA or DHA (unsaturated) for 24 h. The mtDNA/nDNA
ratio increased by 1.4- and 1.9-fold in the presence of 10
and 50 μM EPA compared to the untreated control (Fig.
1A). Also, 50 μM DHA increased the mtDNA copy num-
ber by 1.8-fold compared to control cells (Fig. 1B). In
addition, the mtDNA/nDNA ratio was increased time-
dependently, reaching at 24 h after 50 μM EPA or DHA
treatments (Fig. 1C). However, there were no significant
differences for mtDNA copy number in the presence of
50 μM PA.

Effects of EPA and DHA on expression of genes involved
in mitochondrial biogenesis
To determine the effect of EPA and DHA on expression
of genes involved in mitochondrial biogenesis, the mRNA
levels of PGC-1α, NRF1, and Tfam in C2C12 muscle cells
were measured by real-time quantitative PCR. Cells
were incubated without (control) or with 50 μM of PA,
EPA, or DHA for 24 h. The mRNA levels of PGC-1α,
NRF1, and Tfam increased by 2.3-, 2.0-, and 1.9-fold,
respectively, in the presence of EPA compared with con-
trols (Fig. 2). Furthermore, DHA induced 2.9-, 2.2-, and
2.0-fold increases, respectively, in mRNA levels of PGC-
1α, NRF1, and Tfam compared to control (Fig. 2). In
contrast, PA did not significantly increase in mRNA lev-
els of PGC-1α, NRF1, and Tfam.

Effects of EPA and DHA on PGC-1α promoter activity
To further examine the up-regulated PGC-1α gene ex-
pression by EPA and DHA, we assayed the promoter ac-
vity in C2C12 muscle cells. Cells were treated with 0, 1,
10, or 50 μM EPA or DHA for 40 h. The PGC-1α pro-
moter activity in the presence of 10 and 50 μM EPA in-
creased by 2.0- and 2.5-fold, respectively, compared to
control. Also, 50 μM DHA increased PGC-1α promoter

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Fig. 1. Effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on mtDNA copy number in C2C12 muscle cells.
Differentiated C2C12 muscle cells were treated with 1% bovine serum albumin serum-free medium alone (control), 50 μM palmitate
(PA) or 1, 10, and 50 μM EPA (A) or DHA (B) for 24 h. Cells were time-dependently exposed to 50 μM PA, EPA, or DHA for 6,
16, and 24 h (C). mtDNA copy number was measured by real-time quantitative PCR. Values are expressed as mean±SD (n=3)
of three independent experiments. *P<0.05 and **P<0.01 compared to control.
Mitochondrial biogenesis is a noticeable response of skeletal muscle to a variety of physiological conditions (15,16). There are several food components that have reported to induce mitochondrial biogenesis such as resveratrol (17), green tea polyphenols (18), and curcumin (19). Specifically, resveratrol increased the mitochondrial mass and mitochondrial DNA content, and the mRNA expression of NRF1, Tfam, and PGC-1α in endothelial cells. Also, green tea polyphenols increased mtDNA copy number and the mRNA expression of PGC-1α and Tfam in renal of rats. Curcumin has been shown to increase the mitochondrial DNA copy number and PGC-1α deacetylation in skeletal muscle during endurance training of rats.

EPA and DHA intake from marine sources has been reported to have diverse health benefits that include hypolipidemic effects, improved insulin sensitivity and fatty acid oxidation (20-22). While evidence supports a role for ω-3 PUFA in stimulating the expression of genes encoding regulatory factors for mitochondrial biogenesis and oxidative metabolism in adipose tissue (12), there is limited evidence evaluating the effects of EPA and DHA on muscle mitochondrial biogenesis.

To investigate the effects of EPA and DHA on mtDNA replication in C2C12 muscle cells, the mtDNA copy number was measured by real-time quantitative PCR. The EPA and DHA doses used in this study (1—50 μM) were non-cytotoxic for C2C12 cells, as previously demonstrated (23). EPA and DHA treatment significantly increased mtDNA copy number in skeletal muscle cells. These results agree with a previous study showing that EPA promoted elevated mtDNA copy number in inguinal and brown adipocytes (24). Thus, it can be postulated that EPA and DHA may enhance mitochondrial replication in skeletal muscle cells.

To understand the underlying mechanism of mitochondrial biogenesis by EPA and DHA, we investigated the mRNA levels of genes involved in mitochondrial biogenesis such as PGC-1α, NRF1, and Tfam in C2C12 cells. We found that both EPA and DHA significantly elevated mRNA levels of PGC-1α, NRF1, and Tfam compared to control and PA. PGC-1α serves a major integrative role in the transcriptional regulatory cascade upstream of mitochondrial biogenesis (25). A previous study showed that DHA up-regulated PGC-1α gene expression in 3T3-L1 cells (12). In addition, Gerhart-Hines et al. (26) reported that fasting induced PGC-1α deacetylation in
skeletal muscle and that SIRT1 deacetylation of PGC-1α is required to activate mitochondrial fatty acid oxidation genes. Activated PGC-1α enhances NRF1 and NRF2 expression and increases Tfam expression. This result indicates a link between the nucleus and mitochondria by directly regulating mtDNA replication and transcription (4-6). Previously it was reported that conjugated linoleic acid isomers up-regulated PGC-1α, NRF1, and Tfam expression in C2C12 cells (27). Also, a recent study showed that ω-3 PUFA from cod fish oil induced the activation of PGC-1α and β3 in rat skeletal muscles (28). In our study, EPA and DHA increased the expression of PGC-1α, NRF1, and Tfam genes in C2C12 cells. It is assumed that EPA and DHA directly modulate the expression of genes involved in muscle mitochondrial biogenesis.

Enhancement of the PGC-1α mRNA level may be derived from increased transcription and/or promotion of mRNA stability. To distinguish between these possibilities, the effects of EPA and DHA on PGC-1α promoter activity were examined in C2C12 cells. PGC-1α promoter activity was elevated by both EPA and DHA treatment in a dose-dependent manner, in parallel with the altered mRNA expression. In a previous study, we showed that EPA and DHA increased expression of mitochondrial uncoupling protein 3 (UCP3) mRNA and enhanced UCP3 expression. In a previous study, we showed that ω-3 PUFA from cod fish oil induced the activation of PGC-1α and β3 in rat skeletal muscles (28). In our study, EPA and DHA increased the expression of PGC-1α, NRF1, and Tfam genes in C2C12 cells. It is assumed that EPA and DHA directly modulate the expression of genes involved in muscle mitochondrial biogenesis.

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This study investigated the effects of EPA and DHA on mitochondrial biogenesis in C2C12 cells. The results showed that both EPA and DHA increased mtDNA copy number and up-regulated mRNA expression of genes involved in mitochondrial biogenesis, such as PGC-1α, NRF1, and Tfam in C2C12 cells. Moreover, both EPA and DHA increased PGC-1α promoter activity in parallel with mRNA expression. These findings suggest that EPA and DHA might regulate muscle mitochondrial biogenesis, potentially through mtDNA replication and PGC-1α promoter activation in C2C12 cells.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM. 2002. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. Nature 418: 797-801.
2. Scarpulla RC. 2008. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol Rev 88: 611-638.
3. Handschin C, Spiegelman BM. 2008. Hypothesis article the role of exercise and PGC1α in inflammation and chronic disease. Nature 454: 463-469.
4. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98: 115-124.
5. Virbasius JV, Scarpulla RC. 1994. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. Proc Natl Acad Sci USA 91: 1309-1313.
6. Parisi MA, Clayton DA. 1991. Similarity of human mitochondrial transcription factor 1 to high mobility group proteins. Science 252: 965-969.
7. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD. 2000. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 71: 179S-188S.
8. Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Pérusse L, Vohl MC. 2014. SREBF1 gene variations modulate insulin sensitivity in response to a fish oil supplementation. Lipids Health Dis 13: 152.
9. Anderson EJ, Thayne KA, Harris M, Shaikh SR, Darden TM, Lark DS, Williams JM, Chitwood WR, Kypson AP, Rodriguez E. 2014. Do fish oil omega-3 fatty acids enhance antioxidant capacity and mitochondrial fatty acid oxidation in human atrial myocardium via PPARγ activation?. Antioxid Redox Signal 21: 1156-1163.
10. Thorsdottir I, Tomasson H, Gunnarsdottir I, Gisladottir E, Kiely M, Parra MD, Bandarra NM, Schaafsma G, Martínez JA. 2007. Randomized trial of weight-loss-diet for young adults varying in fish and fish oil content. Int J Obes 31: 1560-1566.
11. Ramel A, Parra D, Martínez JA, Kiely M, Thorsdottir I. 2009. Effects of seafood consumption and weight loss on fasting leptin and ghrelin concentrations in overweight and obese young adults. Eur J Nutr 48: 107-114.
12. Flachs P, Horakova O, Brauner P, Rosmeisl M, Pecina P, Fransen-van Hal N, Ruzickova J, Sponarova J, Drahota Z, Vlcek C, Keijer J, Houdek J, Kopecky J. 2005. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce β-oxidation in white fat. Diabetologia 48: 2365-2375.
13. Jeng JY, Lee WH, Tsai YH, Chen CY, Chao SY, Hsieh RH. 2009. Functional modulation of mitochondria by eicosapentaenoic acid provides protection against ceramide toxicity to C6 glioma cells. J Agric Food Chem 57: 11455-11462.
14. Rozen S, Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol
15. Freyssenet D, Berthon P, Denis C. 1996. Mitochondrial biogenesis in skeletal muscle in response to endurance exercises. *Arch Physiol Biochem* 104: 129-141.
16. Hood DA, Takahashi M, Connor MK, Freyssenet D. 2000. Assembly of the cellular powerhouse: current issues in muscle mitochondrial biogenesis. *Exerc Sport Sci Rev* 28: 68-73.
17. Csiszar A, Labinskyy N, Pinto JT, Ballabh P, Zhang H, Losonczy G, Pearson K, de Cabo R, Pacher P, Zhang C, Ungvari Z. 2009. Resveratrol induces mitochondrial biogenesis in endothelial cells. *Am J Physiol Heart Circ Physiol* 297: H13-H20.
18. Rehman H, Krishnasamy Y, Haque K, Thurman RG, Lemasters JJ, Schnellmann RG, Zhong Z. 2013. Green tea polyphenols stimulate mitochondrial biogenesis and improve renal function after chronic cyclosporin A treatment in rats. *PLoS One* 8: e65029.
19. Ray Hamidie RD, Yamada T, Ishizawa R, Saito Y, Masuda K. 2015. Curcumin treatment enhances the effect of exercise on mitochondrial biogenesis in skeletal muscle by increasing cAMP levels. *Metabolism 64*: 1334-1347.
20. Elvevoll EO, Eilertsen KE, Brox J, Dragnes BT, Falkenberg P, Olsen JO, Kirkhus B, Lamglait A, Østerud B. 2008. Saturated fatty acids: hyperlipidemic and antiatherogenic effects of taurine and n-3 fatty acids. *Atherosclerosis 200*: 396-402.
21. Lombardo YB, Hein G, Chicco A. 2007. Metabolic syndrome: effects of n-3 pufas on a model of dyslipidemia, insulin resistance and adiposity. *Lipids 42*: 427-437.
22. Madsen L, Rustan AC, Vaagenes H, Berge K, Dyroy E, Berge RK. 1999. Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. *Lipids 34*: 951-963.
23. Lee MS, Kim IH, Kim Y. 2013. Effects of eicosapentaenoic acid and docosahexaenoic acid on uncoupling protein 3 gene expression in C2C12 muscle cells. *Nutrients 5*: 1660-1671.
24. Zhao M, Chen X. 2014. Eicosapentaenoic acid promotes thermogenic and fatty acid storage capacity in mouse subcutaneous adipocytes. *Biochem Biophys Res Commun 450*: 1446-1451.
25. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. 2000. Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest 106*: 847-856.
26. Gerhart-Hines Z, Rodgers JT, Bare O, Liner C, Kim SH, Mostoslavsky R, Alt FW, Wu Z, Puigserver P. 2007. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. *EMBO J 26*: 1913-1923.
27. Kim Y, Park Y. 2015. Conjugated linoleic acid (CLA) stimulates mitochondrial biogenesis signaling by the upregulation of PPARγ coactivator 1α (PGC-1α) in C2C12 cells. *Lipids 50*: 329-338.
28. Cavaliere G, Trinchese G, Bergamo P, De Filippo C, Mattace Raso G, Gifuni G, Putti R, Moni BH, Canani RB, Meli R, Mollica MP. 2016. Polyunsaturated fatty acids attenuate diet induced obesity and insulin resistance, modulating mitochondrial respiratory uncoupling in rat skeletal muscle. *PLoS One 11*: e0149033.
29. Siculella L, Sabetta S, Damiano F, Giudetti AM, Gnoni GV. 2004. Different dietary fatty acids have dissimilar effects on activity and gene expression of mitochondrial tricarboxylate carrier in rat liver. *FEBS Lett 578*: 280-284.