Leucine as regulator of mitochondrial biogenesis via modulating PGC-1 α -targeting microRNAs in C2C12 myotubes

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Ph.D. Dissertation

Leucine as regulator of mitochondrial biogenesis via modulating PGC-1α-targeting microRNAs in C2C12 myotubes

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Abstract

Leucine (Leu) has been known to induce mitochondrial biogenesis through the activation of PGC-1α. Studies suggest that miR-494-3p, miR-696, and miR-761 play a role in this process by targeting PGC-1α, although it remains unclear whether nutrient stimulation affects their expression. Therefore, our study focused on the effect of Leu on these miRNA functions and how it affected mitochondrial biogenesis. Following 5 days of differentiation, C2C12 myotubes were treated with Leu (1 or 3 mM) in Leu-free DMEM without serum for 24-h. After treatment, the cells were harvested and DNA, RNA, and whole protein fraction were isolated for downstream analyses. Myotubes treated with 3 mM Leu increased mitochondrial DNA content, although no changes in OXPHOS proteins were observed. In addition, Leu treatments downregulated both miR-494-3p and miR-696 levels. When miR-494-3p and miR-696 levels were overexpressed, PGC-1α and TFAM genes and protein expressions were downregulated, although Leu treatment could stimulate PGC-1α mRNA even in miRNAs overexpression condition. Furthermore, a main effect of Leu treatment was found on mtDNA content, which might indicate a protective role of Leu from the overexpression of miRNAs in C2C12 myotubes. These results suggest that Leu regulates mitochondrial biogenesis possibly through modulation of PGC-1α-targeting miRNAs in C2C12 myotubes.

Introduction

Several studies have demonstrated that small-size nutrients are beneficial for skeletal muscle development by modulating glucose metabolism, fatty acid oxidation, protein synthesis, and other cellular processes (Efeyan et al., 2015). For example, branched-chain amino acid leucine has emerged as a favorite amongst athletes and recreational exercisers to promote protein synthesis and muscle growth (Vaughan et al., 2013). Moreover, leucine induces mitochondrial biogenesis through diverse mechanisms that converge in the activation of peroxisome proliferator-activated γ (PPARγ)-receptor coactivator 1 α (PGC-1α), leading to enhanced transcriptional activity and mitochondrial biogenesis (Schnuck et al., 2016).

It has been well established that nutrients can regulate the expression of protein-coding genes. However, growing evidence has accumulated supporting a role for nutrients in the regulation of miRNA. Within the vast family of miRNAs, we centered our attention on the study of miR-494-3p, miR-696, and miR-761 referred as exercise-responsive miRNAs due to their typical response after physical activity. Although discovered separately, they respond to similar stimuli and share target genes, with PGC-1α as one of the most relevant, thus regulating major cellular processes including mitochondrial biogenesis, fatty acid oxidation, and apoptosis (Aoi et al., 2010; Yamamoto et al., 2012; Long et al., 2013). Some studies suggest that miR-494-3p, miR-696, and miR-761 play a role in these processes by targeting PGC-1α, even though it remains unclear whether nutrient stimulation affects their expression. Therefore, the present study aimed to elucidate the effect of leucine treatment on the expression of these 3 miRNAs and the impact on mitochondrial biogenesis. We hypothesized that: i) leucine regulates the expression of miR-494-3p, miR-696, and miR-761 in skeletal muscle, ii) the regulation of these miRNAs differs between nutrients, and iii) leucine promotes mitochondrial biogenesis partially through the regulation of PGC-1α mediated by these miRNAs.

Methods

This research was divided in two experiments: I) to evaluate the effect of leucine treatment on the regulation of miR-494-3p, miR-696, and miR-761, and II) to elucidate how leucine-induced regulation of miRNAs impacts mitochondrial biogenesis. We began the study by validating the leucine treatment on C2C12 cells. Following 5 days of differentiation, C2C12 myotubes were treated with leucine (Leu; 1 or 3 mM) in serum and leucine-free DMEM for 24-h. Concomitantly,

we supplemented caffeine to another batch of cells, to determine if the effect on miRNAs was similar to leucine treatment. After treatment the cells were harvested and, DNA, RNA, and protein were isolated for immunoblotting and RT-qPCR analyses. Multiple markers were used to assess the effect of leucine on skeletal muscle functions, ranging from the detection of mTOR pathway proteins to mitochondrial markers, including OXPHOS, COXIV, VDAC1, and cytochrome c. Mitochondrial DNA copy number (mtDNA) was also assessed.

To elucidate how leucine-induced regulation of miRNAs impacts mitochondrial biogenesis, gain- and loss-of-miRNA function approaches were used. The transfection with miRNA precursors or antisense sequences was performed on day 2 of differentiation. Forty-eight hours after the transfection, the cells were supplemented with or without leucine (3 mM) for another 24-h. The molecular outcome of both transfection protocols on mitochondrial biogenesis, was assessed through the levels of the miRNAs of interest, as well as mtDNA copy number, mRNA and protein expression of nuclear regulators (PGC-1α and TFAM) were detected.

Results

Leucine Induces Myotube Hypertrophy

Supplementation of leucine at different concentrations (1 and 3 mM) to fully differentiated myotubes resulted in significant hypertrophy *in vitro* (data not shown). In the present study, no changes in the phosphorylation of mTOR were observed after treatment with leucine. On the contrary, phosphorylation of p70S6K, a downstream effector of mTORC1, was significantly increased at 1 mM of leucine concentration (Fig. 1). Additionally, the phosphorylation of 4E-BP1 was increased after treatment with leucine 3 mM ($p < 0.05$). Increased phosphorylation of both proteins is an indicator of protein synthesis stimulation.

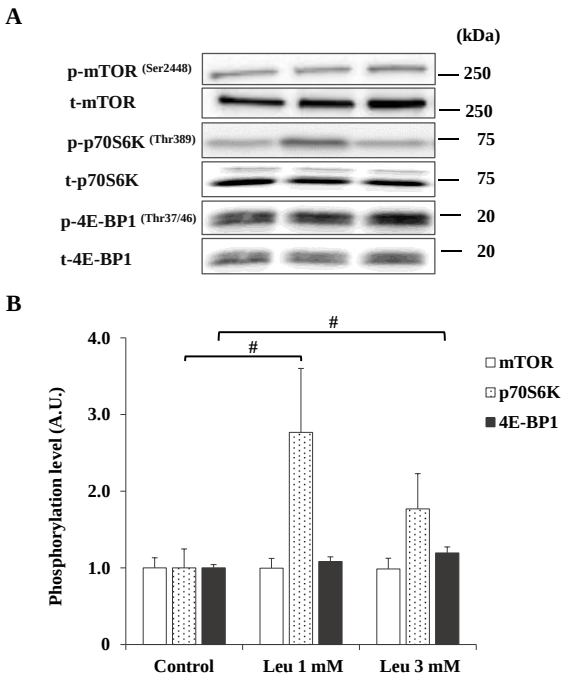

Fig. 1. Phosphorylation levels of mTOR pathway proteins in C2C12 myotubes after 24-h treatment with leucine. (A) Immunoblot images (B) Phosphorylation levels of mTOR and is effectors, p70S6K and 4E-BP1. Values are expressed as mean ± SEM (n = 8/group). # $p < 0.1$, tendency as compared with the control condition by one-way ANOVA with Dunnett's post-hoc test.

Leucine Treatment Increases Mitochondrial Biogenesis Markers in C2C12 Myotubes

Treatment of fully differentiated C2C12 myotubes with 3 mM Leu tended to increase the expression of mtDNA copy number determined by the COXII/UCP2 ratio (p = 0.058, Fig. 2A). On the contrary, no significant changes in the expression of the oxidative phosphorylation (OXPHOS) Complexes I-V was observed (Fig. 2B, 2C). Similarly, leucine treatment did not affect the expression levels of mitochondrial proteins VDAC1, COXIV, and cytochrome (data not shown).

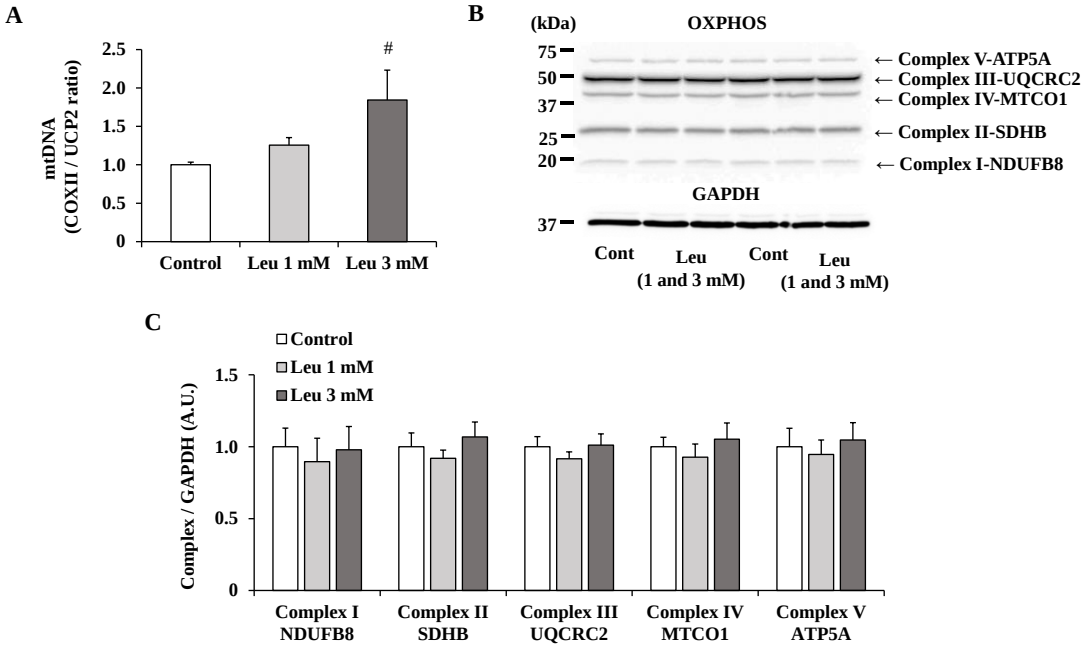

Fig. 2. Effect of leucine treatment on markers of mitochondrial biogenesis after 24-h treatment with leucine. (A) Mitochondrial DNA copy number after treatment with leucine (1 or 3 mM) (B) Immunoblot images of oxidative phosphorylation (OXPHOS) proteins. (C) Expression levels of OXPHOS Complexes I-V. Values are expressed as mean ± SEM (n = 5-8/group). # $p < 0.1$, tendency as compared with the control condition by one-way ANOVA with Dunnett's post-hoc test.

Leucine Downregulates miR-494-3p and miR-696 expression in Myotubes

Regarding the miRNA's expression profile, miR-494-3p levels tended to be lower in both 1- and 3-mM leucine-treated groups (p = 0.096 and 0.084, respectively; Fig. 3A). A similar trend was also observed in the miR-696 level, but the miR-696 expression significantly decreased in the 3 mM leucine group ($p < 0.05$). Overall, there was a strong, positive linear correlation between miR-494-3p and miR-696 levels in C2C12 myotubes ($r = 0.503$, $p < 0.05$, $n = 24$; Fig. 3B). The results summarized in Figure 3B might indicate a cooperative effect between these two miRNAs, independent of the treatment applied. On the contrary, miR-761 levels were not affected by leucine

treatment. Furthermore, caffein treatment had no effect on the expression level of the 3 miRNAs (data not shown).

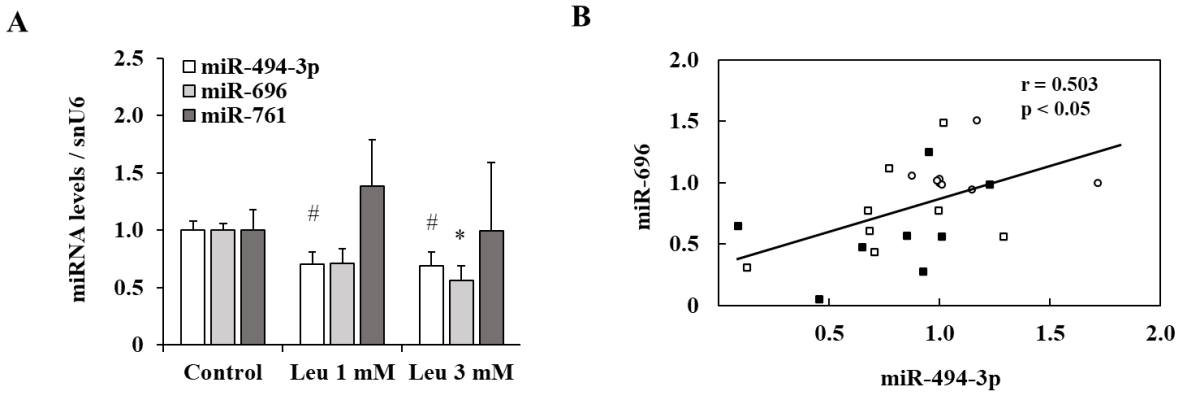

Fig. 3. Fold change in miRNAs after 24-h treatment with leucine. (A) Relative expression levels of miR-494-3p, miR-696 and miR-761 after leucine treatment. (B) Correlation between miR-494-3p and miR-696 expression levels in Control (○), 1 mM Leu (□), 3 mM Leu (■) (n = 8/group, total n = 24). Pearson's correlation coefficient was obtained across all the data, independent of the Leu treatment applied ($r = 0.503$ and $p < 0.05$). Values in (A) and (C) are expressed as mean ± SEM. *: $p < 0.05$, different from the control group, #: $p < 0.1$, tendency as compared with the control condition by one-way ANOVA with Dunnett's post-hoc test.

Effect of miR-494-3p and miR-696 Overexpression on Mitochondrial Biogenesis

Two-way ANOVA indicated a significant main effect of transfection in miR-494-3p and miR-696 expressions ($p < 0.05$, Fig. 4A). The expression level of both miR-494-3p and miR-696 was significantly higher in these miRNAs-transfected cells (abbreviated as miRs OE) than in non-transfected cells ($p < 0.05$). To examine the effect of miR-494-3p and miR-696 overexpression on mitochondrial content, we quantified the mtDNA copy numbers. There was a significant main effect of treatment on mtDNA copy number ($p < 0.05$, Fig. 4B). Compared to the (-) leucine cells, mtDNA copy number was significantly greater in leucine-treated cells ($p < 0.05$). Moreover, two-way ANOVA revealed significant main effects of transfection and treatment in PGC-1α mRNA expression ($p < 0.05$, Fig. 4C). The mRNA expression of PGC-1α was significantly lower in miRs OE than in control cells, whereas the value in Leu-treated cells was significantly higher than in (-) leucine cells. A similar trend, except for the main effect of treatment, was observed in PGC-1α protein expression and TFAM mRNA and protein expressions (Fig. 4D-F). These results indicate that miR-494-3p and miR-696 target PGC-1α while presenting a relationship between leucine treatment, miR-494-3p and miR-696, and PGC-1α in skeletal muscle.

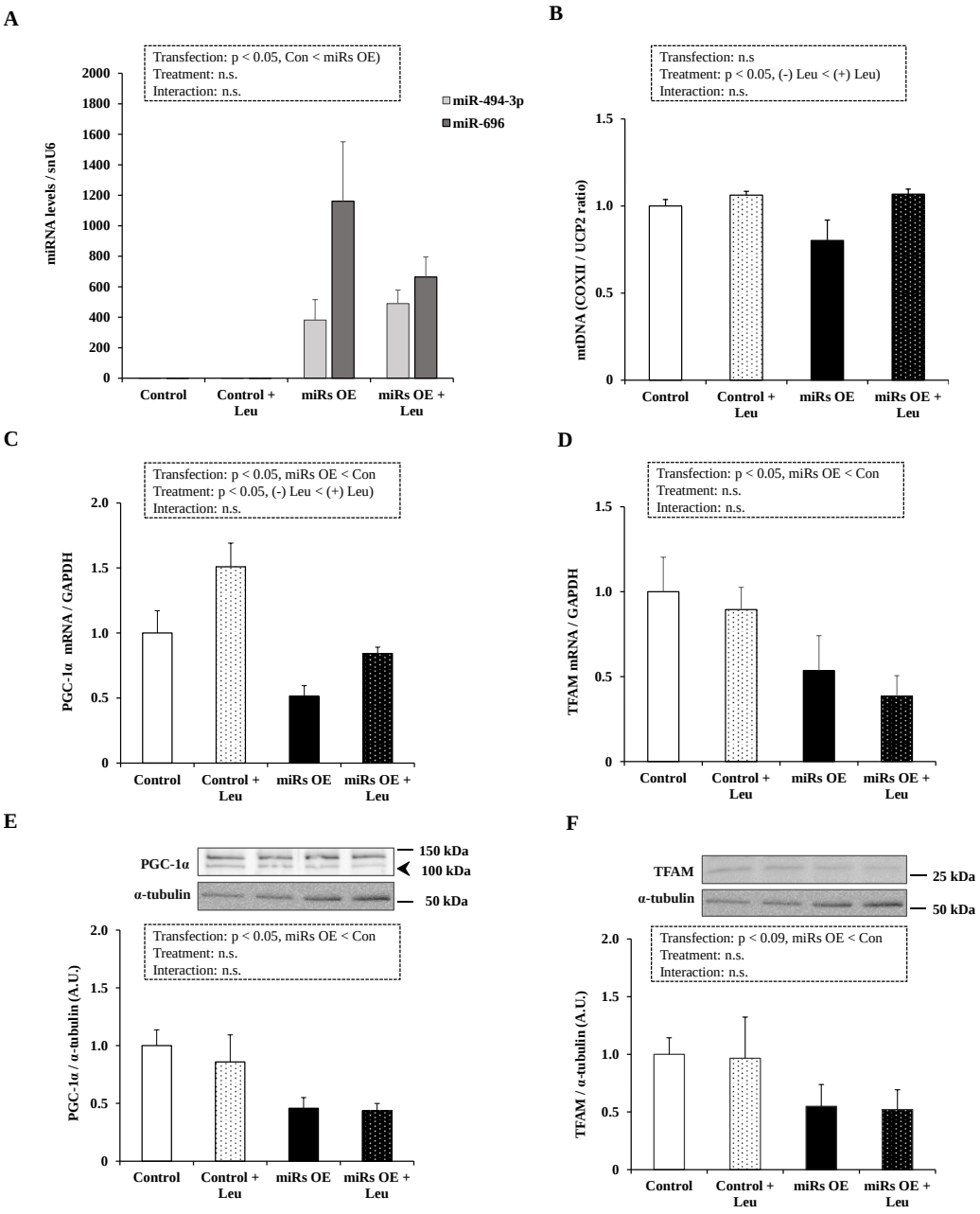

Fig. 4. Effect of miR-494 and miR-696 overexpression on mitochondrial biogenesis. (A) Expression levels of 494-3p and miR-696 were determined by the comparative method $2^{-\Delta\Delta Ct}$ using snU6 as an internal control. (B) Mitochondrial DNA copy number (mtDNA) was determined in cells double transfected with miRNA precursors, followed by Leu treatment. (C) mRNA, and (E) protein expression levels of PGC-1α in cells double transfected with miRNA precursors, followed by Leu treatment. The arrowhead (➤) indicates the quantified band (105 kDa). (D) mRNA, and (F) protein expression levels of TFAM in cells double transfected with miRNA precursors, followed by Leu treatment. Values are expressed as mean ± SEM (n = 3/group). The main effects of miRNA overexpression (miRs OE) and Leu treatment were determined by two-way ANOVA test.

Effect of miR-494-3p and miR-696 Suppression on Mitochondrial Biogenesis

Contrary to the overexpression experiment, two-way ANOVA analysis showed no main effects of the transfection with miR-494-3p and miR-696 inhibitors or leucine treatment on

miRNA levels (Fig. 5A), PGC-1α mRNA and protein (Fig. 5C, 5E), as well as TFAM mRNA and protein expression (Fig. 5D, 5F). In correspondence with the overexpression experiment, mtDNA copy number was significantly greater in leucine-treated cells, compared to the (-) leucine cells (Fig. 5B, $p < 0.05$). These results might indicate the presence of a leucine-induced positive effect on mitochondrial biogenesis, with mtDNA as a sensitive marker to leucine treatment.

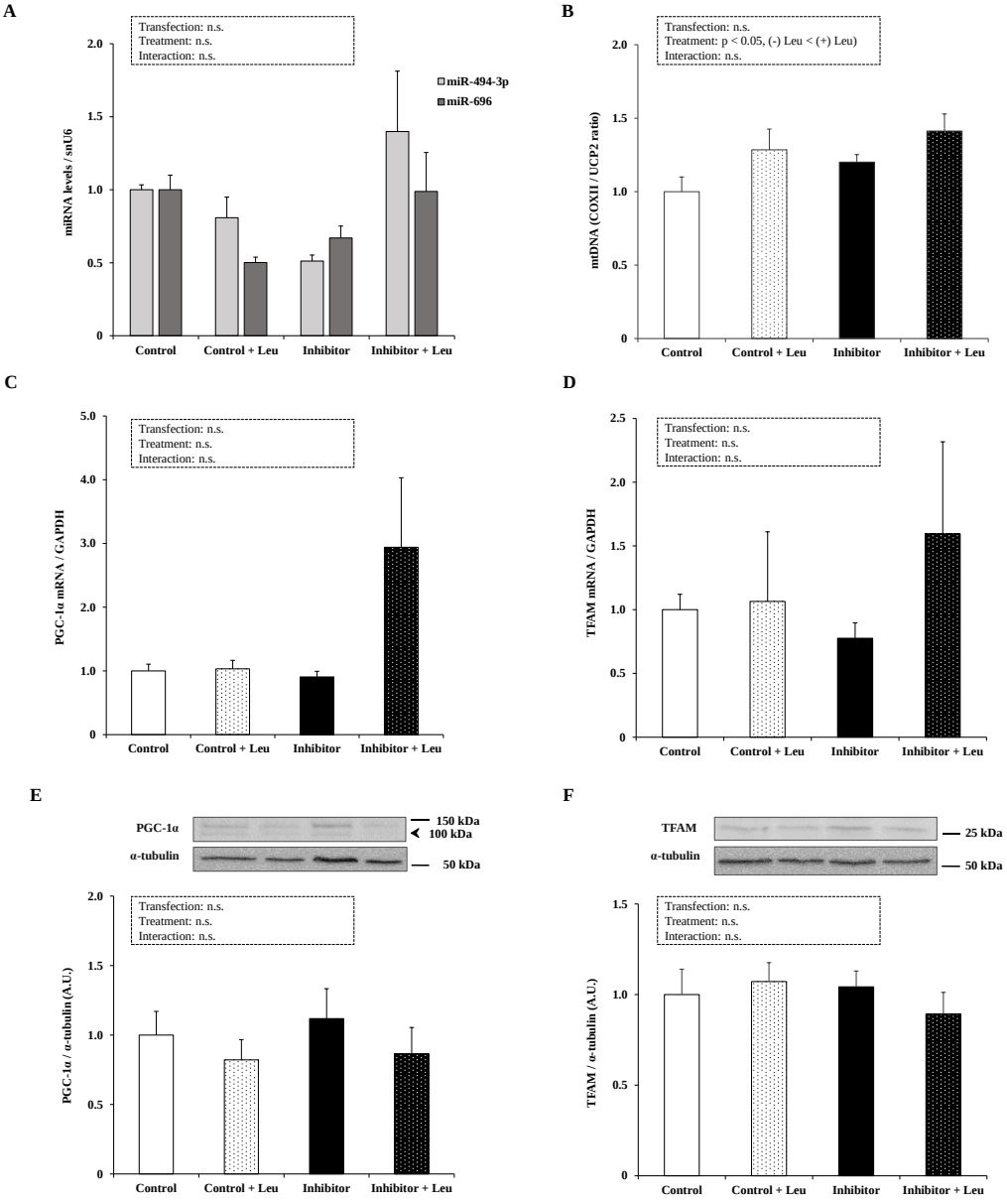

Fig. 5. Effect of miR-494 and miR-696 inhibition on mitochondrial biogenesis. (A) Expression levels of 494-3p and miR-696 were determined by the comparative method $2^{-\Delta\Delta Ct}$ using snU6 as an internal control. (B) Mitochondrial DNA copy number (mtDNA) was determined in cells double transfected with miRNA inhibitors, followed by Leu treatment. (C) mRNA, and (E) protein expression levels of PGC-1α in cells double transfected with miRNA inhibitors, followed by Leu treatment. The arrowhead (➤) indicates the quantified band (105 kDa). (D) mRNA, and (F) protein expression levels of TFAM in cells double transfected with miRNA inhibitors, followed by Leu treatment. Values are expressed as mean ± SEM (n = 4-5/group). The main effects of miRNA inhibition and Leu treatment were determined by two-way ANOVA test.

Discussion

Nutrients constitute an important factor in the regulation of skeletal muscle metabolism. Among the BCAAs, leucine has become a popular dietary supplement and the main component of many ergogenic products (Vaughan et al., 2013). The evidence points towards a key regulatory role of leucine in gene transcription and translation. It is well established that leucine is capable of activating the mRNA translational machinery through the regulation of mTOR, which serves as a convergence point for leucine-mediated effects on mRNA translation initiation and represents an interesting molecular target for the prevention of muscle loss (van Loon, 2012). In the present study, we evaluated mTOR activity through its phosphorylation on residue Ser2448 following 24 h of treatment with two different doses of leucine (1 or 3 mM). Although no differences in mTOR were observed, the phosphorylation levels of the downstream targets p70S6K and 4E-BP1 were augmented, which is in concordance with the observed increase in cell diameter after treatment with leucine (Fig. 1).

Beyond the role in protein synthesis stimulation, leucine has been associated with activation of SIRT1 signaling, suppression of insulin resistance, and prevention against metabolic disorders in a diet-induced obesity animal model (Li et al., 2012). Recently, it was shown that leucine induced a metabolic shift from glycolytic towards OXPHOS in human and murine skeletal muscle cells and also in some models of cancer (Viana et al., 2019). In skeletal muscle, SIRT1 deacetylates PGC-1α to induce the expression of genes controlling mitochondrial biogenesis and fatty acid oxidation to maintain energy homeostasis (Liang et al., 2014). Here we found mtDNA content increased after treatment with leucine 3 mM (Fig. 3). This finding suggests a mild effect of leucine treatment on mitochondrial biogenesis.

In the last three decades, a novel regulatory mechanism of gene expression has been discovered. Small non-coding regulatory RNAs termed miRNAs have been found associated with virtually all cellular processes. Recent studies have suggested that several miRNAs could also function as modulators of myogenesis, muscle mass, and nutrient metabolism in skeletal muscle, thus raising questions regarding the association of miRNAs with exercise-induced physiological changes, muscular pathogenesis, and age-related muscle dysfunction (Aoi, 2015). Understanding the mechanisms by which nutrients, especially leucine, can alter metabolic signaling through the miRNA network will be of great value for the development of effective nutritional and/or pharmacological interventions to prevent and/or treat chronic metabolic diseases.

Diverse dietary components such as amino acids, carbohydrates, fatty acids, and vitamins have been found to affect the expression profile of miRNAs or their function. Particularly, leucine and essential amino acids have been shown to regulate miRNA expression in skeletal muscle (Chen et al., 2013). In the present study, a downregulation of the exercise-responsive miRNAs miR-494-3p and miR-696 was observed after treatment with leucine (Fig. 3A). These miRNAs' expressions were also correlated, suggesting coordinated functions in skeletal muscle. Next, the gain-of-function approach confirmed the regulation of the PGC-1α by miR-494-3p and miR-696 in myotubes transfected with these miRNAs' precursors (Fig. 4C, 4E), which impacted the expression of nuclear factor TFAM, acting downstream of PGC-1α (Fig. 5D, 5F). However, we only visualized a partial recovery of PGC-1α gene expression associated with leucine treatment. In both, gain- and loss-of-function experiments, leucine treatment showed increased mtDNA copy numbers (Fig. 4B, 5B). These facts indicate that leucine might promote mitochondrial biogenesis partially through the regulation of PGC-1α-targeting miRNAs.

Regarding the control of miRNA expression, diverse regulatory levels have been proposed. At the molecular level, beside the mutual control among miRNAs and their mRNA targets, observations indicate the existence of the so-called "miRNA sponges". These consist of long non-coding RNAs (lncRNAs), pseudogenes, circular RNAs, and mRNAs that can scavenge specific miRNAs at their seed site, preventing the silencing complex (RISC) to bind their target mRNAs, or competing with miRNAs for binding to the specific mRNAs (Ye et al., 2015). Nevertheless, the exact relationship between nutrients and miRNAs biogenesis in skeletal muscle is not well documented. Here we summarized the possible two mechanisms reported until now, along with our findings. Under high-nutrient conditions (amino acid- or glucose-sufficient), activation of mTORC1 increases the levels of ubiquitin E3 ligase Mdm2 that ubiquitinates and targets the miRNA-processing enzyme Drosha for proteasomal-dependent degradation, this leading to a global decrease of miRNA biogenesis (Ye et al., 2015). Herein, we determined the phosphorylation of mTOR and its main downstream effector proteins including p70S6K and 4E-BP1. The two proteins were significantly increased by leucine treatment, indicating possible activation of the pathway; however, this is not sufficient to explain the observed downregulation. There is another mechanism that is related to the epigenetic regulation of gene expression by nutrients (Keating and El-Osta, 2015). Accordingly, amino acids and other metabolites are catabolized to acetyl-CoA, the essential acetyl group donor to lysine-acetylation reaction. Because histone acetylation is

ubiquitously associated with open-chromatin and gene expression, amino acid catabolism provides a metabolic link to epigenetic regulation (Phang et al., 2013). Data shows that miRNAs are not only regulated by multiple epigenetic mechanisms but can also repress the expression of epigenetic factors (Quintanilha et al., 2017). Moreover, DNA methylation and histone modifications often cooperatively regulate miRNA expression (Bianchi et al., 2017). This leaves open a whole new research field of study to elucidate the regulation of miRNA biogenesis under different nutritional conditions.

There are several limitations of this study that should be addressed in future research. First, the study focused solely on the effect of leucine treatment of the miRNAs of interest to examine the hypothesized underlying regulatory mechanism. Second, the difficulties with determining the significance of miRNA changes need to be taken into consideration since each miRNA has several targets and the resulting changes are often contradictory. Herein, only two target genes, PGC-1α and TFAM were evaluated, therefore other cellular changes might have been missed. Finally, no interactions were found between leucine treatment and miRs OE, possibly due to the small sample size used.

Conclusion

In conclusion, leucine promoted protein synthesis through activation of the mTOR pathway, as well as mitochondrial biogenesis. Furthermore, the expression of miR-494-3p and miR-696 was downregulated after leucine treatment in C2C12 myotubes. Caffeine, on the other hand, had no effect on miRNA levels. These results suggest the presence of a leucine-specific miRNA profile. Finally, we observed that miR-494-3p and miR-696 overexpression negatively regulate PGC-1α mRNA, an effect that is partially restored by leucine treatment. The present study constitutes the first report of a nutrient, especially leucine, regulating mitochondrial biogenesis through exercise-responsive miRNAs. Understanding the mechanisms by which nutrients, especially leucine, can alter metabolic signaling through the miRNA network will be of great value for the development of effective nutritional and/or pharmacological interventions to prevent and/or treat chronic metabolic diseases.

References

Aoi W, Naito Y, Mizushima K, Takanami Y, Kawai Y, Ichikawa H, Yoshikawa T. 2010. The microRNA miR-696 regulates PGC-1α in mouse skeletal muscle in response to physical activity. Am J Physiol - Endocrinol Metab 298: 799-806.

Aoi W. 2015. Frontier impact of microRNAs in skeletal muscle research: A future perspective. Front Physiol 6: 495.

Bianchi M, Renzini A, Adamo S, Moresi V. 2017. Coordinated actions of microRNAs with other epigenetic factors regulate skeletal muscle development and adaptation. Int J Mol Sci 18: 840.

Chen X, Huang Z, Chen D, Yang T, Liu G. 2013. MicroRNA-27a is induced by leucine and contributes to leucine-induced proliferation promotion in C2C12 cells. Int J Mol Sci 14: 14076-14084.

Efeyan A, Comb WC, Sabatini DM. 2015. Nutrient-sensing mechanisms and pathways. Nature 517: 302-310.

Keating ST, El-Osta A. 2015. Epigenetics and metabolism. Circ Res 116: 715–736.

Liang C, Curry BJ, Brown PL, Zemel MB. 2014. Leucine modulates mitochondrial biogenesis and SIRT1-AMPK signaling in C2C12 myotubes. J Nutr Metab 2014: 239750.

Li H, Xu M, Lee J, He C, Xie Z. 2012. Leucine supplementation increases SIRT1 expression and prevents mitochondrial dysfunction and metabolic disorders in high-fat diet-induced obese mice. Am J Physiol - Endocrinol Metab 303: E1234-E1244.

Long B, Wang K, Li N, Murtaza I, Xiao JY, Fan YY, Liu CY, Li WH, Cheng Z, Li PF. 2013. MiR-761 regulates the mitochondrial network by targeting mitochondrial fission factor. Free Radic Biol Med 65: 371-379.

Phang JM, Liu W, Hancock C. 2013. Bridging epigenetics and metabolism: Role of non-essential amino acids. Epigenetics 8: 231-236.

Quintanilha BJ, Reis BZ, Silva Duarte GB, Cozzolino SMF, Rogero MM. 2017. Nutrimiromics: Role of micrornas and nutrition in modulating inflammation and chronic diseases. Nutrients 9: 1168.

Schnuck JK, Sunderland KL, Gannon NP, Kuennen MR, Vaughan RA. 2016. Leucine stimulates PPARβ/δ-dependent mitochondrial biogenesis and oxidative metabolism with enhanced GLUT4 content and glucose uptake in myotubes. Biochimie 128-129: 1-7.

van Loon LJC. 2012. Leucine as a pharmaconutrient in health and disease. Curr Opin Clin Nutr Metab Care 15: 71-77.

Vaughan RA, Garcia-Smith R, Gannon NP, Bisoffi M, Trujillo KA, Conn CA. 2013. Leucine treatment enhances oxidative capacity through complete carbohydrate oxidation and increased mitochondrial density in skeletal muscle cells. Amino Acids 45: 901-911.

Viana LR, Tobar N, Natacha E, Busanello B, Marques AC, Oliveira AG De, Lima TI, Machado G, Castelucci BG, Ramos CD, Brunetto SQ, Silveira LR, Vercesi AE, Consonni SR, Gomes-marcondes MCC. 2019. Leucine-rich diet induces a shift in tumour metabolism from glycolytic towards oxidative phosphorylation, reducing glucose consumption and metastasis in Walker-256 tumour-bearing rats. Sci Rep 9:15529.

Yamamoto H, Morino K, Nishio Y, Ugi S, Yoshizaki T, Kashiwagi A, Maegawa H. 2012. MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcription factor A and Forkhead box j3. Am J Physiol Endocrinol Metab 303: 1419-1427.

Ye P, Liu Yu, Chen C, Tang F, Wu Q, Wang X, Liu CG, Liu X, Liu R, Liu Yang, Zheng P. 2015. An mTORC1-Mdm2-Drosha axis for miRNA biogenesis in response to glucose- and amino acid-deprivation. Mol Cell 57: 708-720.

令和２年７月３１日

学位論文審査報告書（甲）

１．学位論文題目（外国語の場合は和訳を付けること。）

Leucine as regulator of mitochondrial biogenesis via modulating PGC-1α-targeting microRNAs in C2C12 myotubes 骨格筋培養細胞C2C12におけるロイシンによるPGC-1αを標的とするmicro RNAsを介したミトコンドリア生合成の制御

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(2) 氏（ふり）　名（がな） PÉREZ LÓPEZ CLAUDIA（ペレス　ロペス　クラウディア）

３．審査結果の要旨（600～650字）

本論文は栄養基質、とりわけ分岐鎖アミノ酸のロイシン（Leu）が骨格筋細胞のミトコンドリア（Mito）生合成を亢進させることを背景に、その抑制因子として働く可能性がある特定のマイクロRNA（miR-494-3p, miR-696, miR-761）の関与を仮定し、Leuによる新たなMito生合成調節機構の存在について検証した。分化誘導後のC2C12筋管細胞に対してLeuを添加すると筋管の肥大とMito-DNA（mtDNA）のコピー数の上昇が誘導されたが、その際、注目したマイクロRNAのうちmiR-494-3pとmiR-696の発現レベルの抑制が生じることを示した（miR-761は不変）。また、遺伝子導入が比較的難しいC2C12細胞に対して、Leuに挙動を示したmiR-494-3pとmiR-696の一過性過剰発現細胞の作成を成功させ、この細胞におけるPGC-1α遺伝子発現の減少と、mtDNA量ならびに一部の核内転写因子の遺伝子発現の減少傾向も示した。さらに、この過剰発現細胞へのLue添加が、これらの減少の一部を阻止することも示唆した。このように本論文は、骨格筋細胞のMito生合成における特定のマイクロRNAとLeuによる新たな分子調節機序の存在を示唆している。本研究成果はマイクロRNAを標的にした新たな運動・栄養処方に応用が期待される分子基盤研究であることから、審査委員会は本論文が博士（理学）に値すると判断した。（612文字）

４．審査結果 (1) 判　　定（いずれかに○印） （合　格） ・ 不合格

(2) 授与学位 博　士（理　学）