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LETTERS

The effect of CGRP antagonist and exercise training on mitochondrial dynamics in the aorta of male rats

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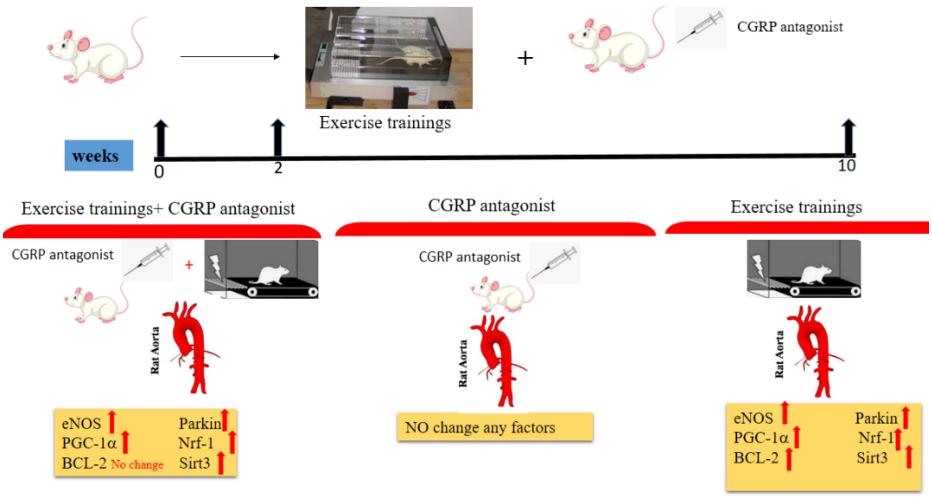
Original Article

ABSTRACT Calcitonin Gene Related Peptide (CGRP) is expressed in the cardiovascular system and demonstrates vasodilatory effects. This study aimed to investigate the effects of exercise training and CGRP antagonism on the expression of genes involved in mitochondrial dynamics in the aorta. Forty-two male rats were randomly divided into six groups (n=7 each): 1) Control; 2) Endurance Training (ET); 3) High-Intensity Interval Training (HIIT); 4) CGRP antagonist (CGRPi); 5) CGRPi-ET; and 6) CGRPi-HIIT. Protein expression was analyzed using Western blotting, while gene expression was quantified via real-time PCR. Both ET and HIIT significantly upregulated the protein levels of eNOS and the gene expression of *Pgc-1α*, *Bcl-2*, *Nrf-1*, *Sirt3*, *Parkin*, and *eNOS*. The increase in *Bcl-2* expression induced by ET was attenuated by CGRPi in the groups receiving the combination of CGRPi and ET (P = 0.02). While CGRP is recognized as a vasodilator, our results suggests that CGRPi does not affect eNOS expression, indicating that CGRP exerts vasodilatory effects through mechanisms other than eNOS in the aorta. Furthermore, CGRPi does not negatively affect genes associated with mitochondrial dynamics, indicating its potential safety for use in migraine treatment and other conditions.



Keywords: Olcegepant, CGRP, eNOS, Pgc-1α, Bcl-2

INTRODUCTION

Alterations in endothelial function contribute to an increased risk of cardiovascular diseases such as ischemic and hemorrhagic strokes, angina pectoris, and myocardial infarction, as observed in various studies comparing migraine sufferers with non-migraineurs.[1-3] Under physiological conditions, CGRP modulates vascular responses and protects against injury. Thus, CGRP serves a cardioprotective role by inhibiting oxidative stress and the proliferation of vascular smooth muscle cells (VSMCs) in response to vascular injury.[4,5] Also, elevated nitric oxide (NO) level induces CGRP expression and neuronal nitric oxide synthase (nNOS).[6]

NO is an essential endogenous signaling molecule that plays significant roles in vascular function and mitochondrial biology. eNOS has been reported to enhance the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and nuclear respiratory factor-1 (Nrf-1), thereby promoting mitochondrial biogenesis.[7,8] Increased mitochondrial biogenesis may ameliorate pathology and slow disease progression in various mitochondrial-associated disorders.[9]

PGC-1α is a primary regulator of mitochondrial biogenesis and function; its loss can reduce NO expression.[10,11] Additionally, Sirt3, a target of PGC-1α is involved in neutralizing mitochondrial reactive oxygen species (ROS).[11] Although the association between oxidative stress and the pathophysiology of several diseases, including migraines, is well-established, finding a disorder that is not related to oxidative stress is challenging.

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ROS are normally generated in mitochondria, and excessive ROS can lead to mitochondrial dysfunction, cellular damage, and clinical disorders [8]. Importantly, oxidative stress and NO levels have been observed to increase during migraine attacks.[7]

CGRP antagonists are considered potential migraine treatments, effectively reducing headache frequency and enhancing patient quality of life. While the efficacy of CGRP antagonism is supported by experimental studies, the underlying mechanisms require further elucidation.[12]

Exercise training enhances mitochondrial biogenesis through mediators such as PGC-1α.[13] Short-term exercise does not affect CGRP levels, while prolonged exercise can increase cardiac CGRP secretion.[14] Although fast- and slow-twitch fibers show no significant changes in CGRP levels after strength and endurance training,[15] elevated ROS levels are known to cause endothelial dysfunction, while CGRP in endothelial cells induces NO-dependent vasodilation.[16]

Nrf-1 and Nrf-2 regulate the expression of electron transport chain subunits, with Nrf-1 expression being modulated by PGC-1α.[17] Sirt3 is predominantly expressed in metabolically active tissues and is upregulated during exercise, also playing a role in regulating energy homeostasis via AMPK and PGC-1α.[18] Sirt3 increases the beta-oxidation of fatty acids and ATP production in the liver, with its expression rises post-exercise in both human and rodent models.[19]

Plasma CGRP levels increase after exercise. While baseline CGRP levels are important for maintaining cardiac function in chronic hypertension, CGRP antagonists do not elevate blood pressure in hypertensive mouse models.[20] An increase in blood CGRP levels in response to exercise in young adults suggests potential effects on physical function.[21] Intravenous CGRP administration has been shown to cause vasodilation and reduce blood pressure in humans. The significant influence of CGRP on cardiovascular function underscores its potential role in cardiovascular health. Blood pressure responses in CGRP knockout mice demonstrate that angiotensin II administration reduces eNOS levels without significant changes in blood pressure.[22] Most reported benefits of CGRP have been derived from laboratory studies, with limited clinical trials available; however, clinical trials for CGRP antagonists may clarify and confirm these relevant effects.

Animal models have confirmed CGRP's protective role against ischemia in cardiac tissue, though the cardiovascular effects of CGRP antagonists, particularly in the aorta, remain less understood. Interestingly, CGRP antagonism does not result in tissue damage.[23] Considering the therapeutic applications of CGRP antagonists in conditions like migraines, hypertension, and heart failure,[24] and the critical role of NO in mitochondrial biogenesis, this study aimed to examine the influences of CGRP antagonism in combination with different exercise training modalities (ET and HIIT) on mitochondrial dynamics, apoptosis, and biogenesis in the rat aorta. We have clearly delineated that our primary aim is to understand the cardiovascular health impacts of CGRP outside of its association with migraine headaches.

MATERIAL AND METHODS

Materials

The CGRP antagonist (BIBN 4096, Cat. No. 4561, Tocris Bioscience), primers (Metabion international AG, Germany), high ROX SYBR green for Real-time PCR (Ampliqon, A325402), RNA extraction kit (Bio Basic, BS1361), cDNA synthesis kit (Parstous, A101162), anti-eNOS antibody (Santa Cruz, sc-376751), and anti-beta actin antibody (Santa Cruz, sc-47778) were obtained from their respective suppliers.

Animals

Forty-two male Wistar rats were utilized for this study. The animals were housed at a temperature of 23±2°C under a 12-hour light/dark cycle with free access to standard chow and water. Following acclimatization, the rats were randomly assigned to six groups (n=7 each): 1) Untreated control (CTL), 2) endurance training (ET) group that performed ET for 6 weeks; 3) High-Intensity Interval Training (HIIT) group that performed HIIT for 6 weeks; 4) CGRP antagonist (CGRPi) administered at 10 mg/kg via intraperitoneal injections daily for the last two weeks; 5) CGRPi-ET; and 6) CGRPi-HIIT. The training regimen finished 24 hours prior to sacrifice. The study duration was six weeks, at the end of which animals underwent a 12-hour overnight fast, were anesthetized with ketamine and xylazine (80/10 mg/kg), and subsequently euthanized. Aortic tissues were excised, washed with cold saline, and subsequently frozen in liquid nitrogen before being stored at -80°C for further quantification. All procedures were approved by the Animal Research Ethics Committee of Kerman University of Medical Sciences (Ethics Committee Permission No. IR.KMU.REC.1399.584).

CGRP antagonist

The CGRP antagonist was administered during the last 14 days of the study through daily intraperitoneal injections (10 mg/kg) to inhibits CGRP functions.[25,26]

Training protocol

Rats adapted to a speed of 15 m/min for 15 minutes over two weeks. To measure VO_{2max}, we utilized the Vmax. Rats completed an incremental exercise test until exhaustion, after which VO_{2max} was calculated. The intensity of exercise training was quantified by monitoring blood lactate levels immediately following exercise with a lactometer (Lactate Scout Company/Code: 37, Germany), with values above 6 mmol/L deemed high intensity [27]. The speed test began with a warm-up at 10 m/min, which gradually increased by 0.3 m/min until exhaustion [15]. Ten two-minute work bouts were conducted daily at approximately 22 m/min, 29 ° slope, interspersed with two-minute rest periods (Table 1), five days a week for six weeks [28, 29]. The control and CGRPi groups remained stationary on the treadmill for each session to establish a stress environment. Details of the endurance training regimen are provided in Table 2 below.

Table 1. High-intensity interval training

Week	1	2	3	4	5	6
Speed (m/min)	15	20	24	24	28	30
Slope (o)	5	10	20	25	29	29

Table 2. Endurance Training

Week	1	2	3	4	5	6
Speed (m/min)	60V max	65%V max	70%V max	70%V max	75%V max	75%V max
Time (min)	20	30	30	40	40	50

Real-time PCR

Total RNA was isolated by EZ-10 spin column RNA extraction kit per the manufacturer's instructions. Approximately 10 mg of the aorta was used for RNA extraction. Then, 100 ng of the isolated RNA was utilized for cDNA synthesis. Real-time PCR was performed using 2X high ROX Master Mix. Each PCR reaction included forward and reverse primers (Table 3), sterile water, and 100 ng cDNA, conducted on the ABI Step One Plus instrument. The thermal cycling involved one initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 20 seconds and 60°C for 30 seconds. A melt curve analysis was subsequently performed, starting from 60°C and increasing by 0.3°C. The expression of each gene was quantified using the $2^{-\Delta\Delta Ct}$ method, normalized to 18S rRNA as a housekeeping gene [30].

Western blotting

Protein extraction was performed used cold RIPA buffer (containing protease inhibitor cocktail, 1 mM phenylmethylsulfonyl fluoride [PMSF], and 1 mM sodium orthovanadate, pH 7.4) and 10 mg of aorta tissue. Homogenization was conducted on ice-cold RIPA buffer using the Hielscher Ultrasound Sonicator, and the homogenate was centrifuged at 15000 rpm, 4 °C for 20 minutes. The supernatant obtained was used for further studies. The total protein in the supernatant was quantified using the Bradford method. For SDS-PAGE, an equal volume of 2X sample buffer was added to each sample and incubated 95°C for 5 minutes, followed by loading 80 micrograms of total protein into each well of a 12 % gel. Protein separation was carried out under constant voltage (120 V) for 75 minutes in Tris-Gly running buffer (pH 8.3). After electrophoresis, proteins were transferred to a Polyvinylidene Difluoride (PVDF) membrane at a constant current of 220 mA in a cold transfer buffer. The membrane was blocked overnight at 4°C with a blocking buffer composed of 5% skim milk in tris-buffered saline with Tween 20. After blocking, the membrane was washed four times for five minutes each. The membrane was then incubated with the primary antibody for two hours, washed four times again, and subsequently incubated with the secondary antibody for an hour and a half, followed by another four washes. The PVDF membranes were treated with Western Lightening Plus ECL for approximately 2 minutes, and the antigen-antibody complex was detected using enhanced chemiluminescence detection film in a dark room. Band densities were analyzed using ImageJ software, with beta-Actin serving as the housekeeping control.[30]

Table 3. The primer' sequences that used in this study to perform Real-time PCR

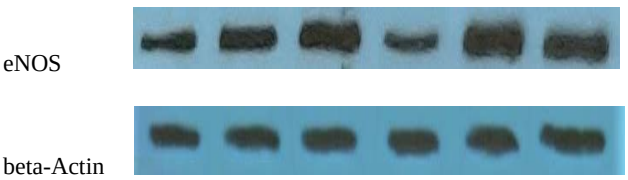
	Gene	Forward	Reverse
1	*Bax*	ATCCAAGACCAGGGTGGCTG	CACAGTCCAAGGCAGTGGGA
2	*Bcl-2*	TATATGGCCCCAGCATGCGA	GGGCAGGTTTGTCGACCTCA
3	*iNOS*	CACCACCCTCCTTGTTCAAC	CAATCCACAACTCGCTCCAA
4	*eNOS*	CGAGATATCTTCAGTCCCAAGC	GTGGATTTGCTGCTCTCTAGG
5	*Nrf-1*	TAGCCCATCTCGTACCATCAC	TTTGTTCCACCTCTCCATCAG
6	*Pgc-1α*	ACCCACAGGATCAGAACAAACC	GACAAATGCTCTTTGCTTTATTGC
7	*Parkin*	CTGGCAGTCATTCTGGAC	CTCTCCACTCATCCGGTTT
8	*Sirt3*	TGCACGGTCTGTCGAAGGTC	TGTCAGGTTTCACAACGCCAG
9	*18S*	GCAATTATTCCCCATGAACG	GGCCTCACTAAACCATCCAA

Statistical analysis

The results obtained from Real-time PCR and Western blotting are presented as mean ± SEM. Data analysis was performed using SPSS software (SPSS Inc., Chicago, IL; Version 20). The normality of the data was assessed using the Shapiro-Wilk test. Upon confirming normal distribution, one-way ANOVA was conducted for group comparisons, followed by pair-wise comparisons using post-hoc Tukey's test, where p-values less than 0.05 were considered statistically significant.

RESULTS

Exercise training (ET and HIIT) significantly upregulated the expression of eNOS protein, as well as *Pgc-1a*, *Bcl-2*, *Nrf-1*, *Sirt3*, *Parkin*, and *eNOS* gene expression compared to the control group ($p < 0.001$) (Figs. 1-3 and Figs. 5-8). *Pgc-1α* levels were reduced by CGRPi in ET-CGRPi and HIIT-CGRPi compared to the ET and HIIT, respectively ($p = 0.032$, $p = 0.019$, respectively) (Fig. 3). The aforementioned genes were also up-regulated compared to the CGRPi group. The expression of *Bax* and *iNOS* genes were not significantly different among the groups (Fig. 4 and 9). CGRPi did not alter either genes or eNOS protein levels. The exercise-induced increase in *Bcl-2* expression was attenuated in the groups receiving combined CGRPi and exercise training (Fig. 5).



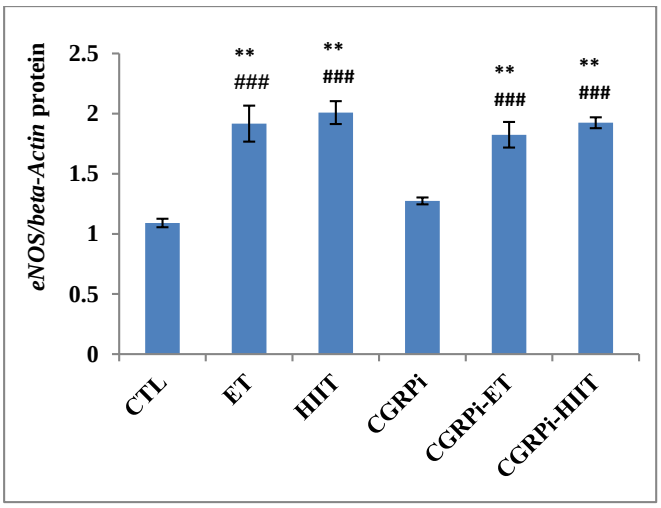


Figure 1. Levels of eNOS protein quantified by western blot across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$

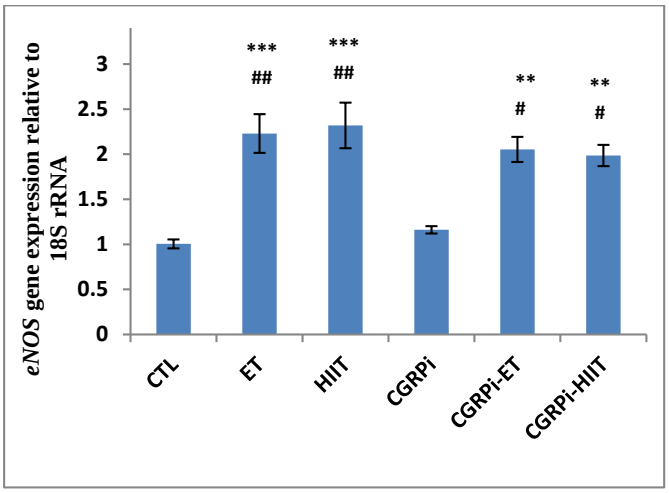


Figure 2. Levels of *eNOS* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

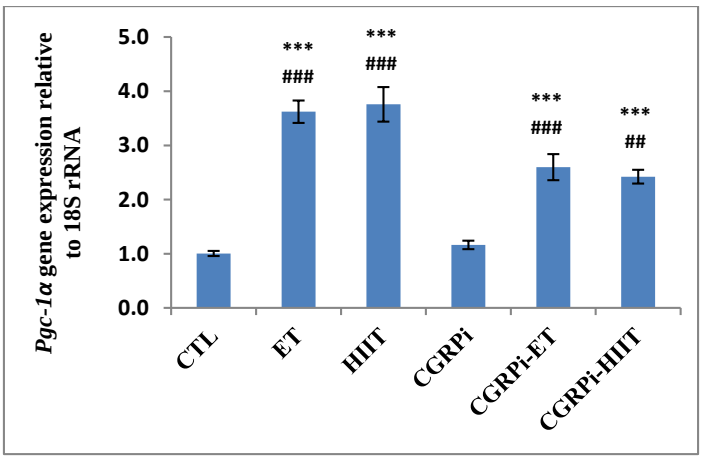


Figure 3. Levels of *Pgc-1α* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

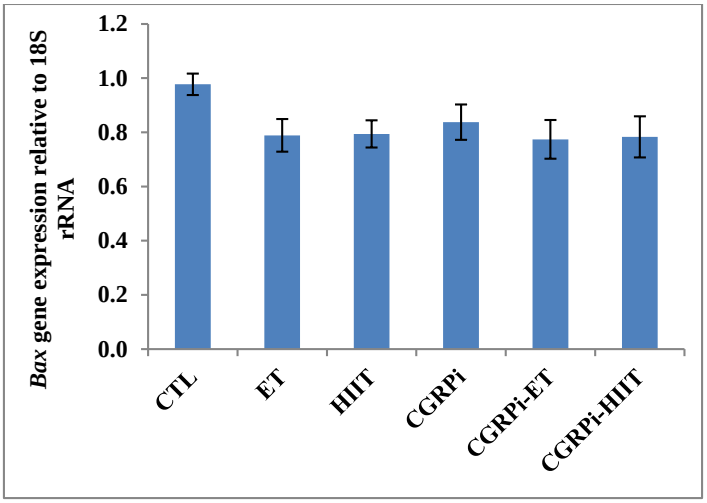


Figure 4. Levels of *Bax* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

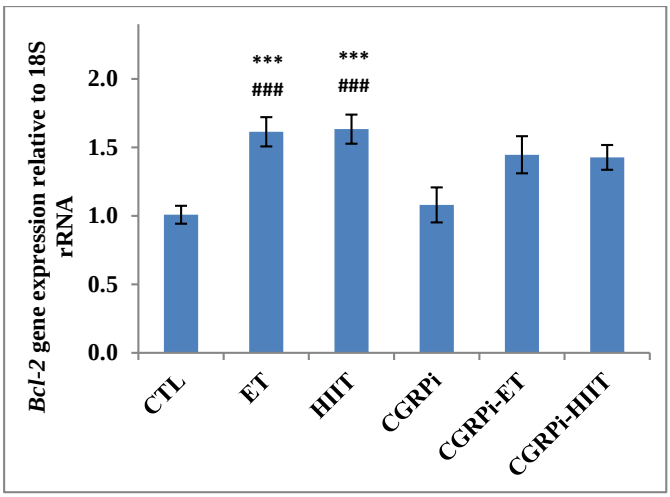


Figure 5. Levels of *Bcl-2* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

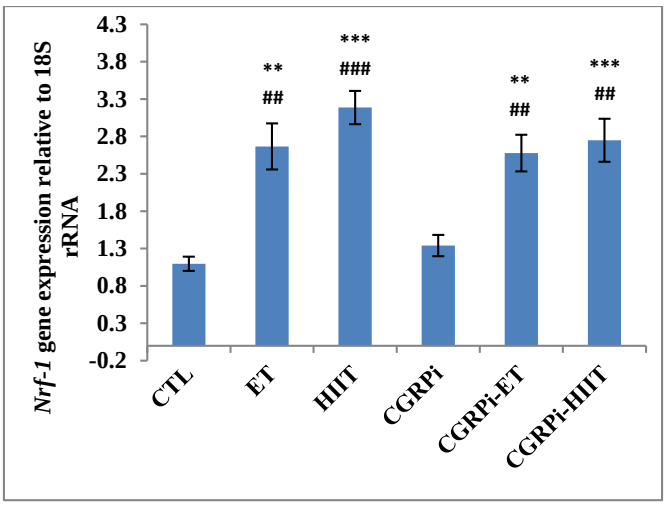


Figure 7. Levels of *Nrf-1* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

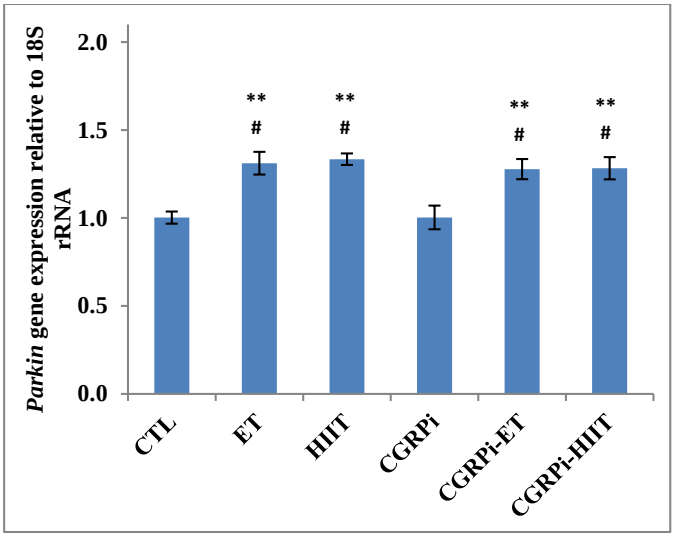


Figure 6. Levels of *Parkin* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

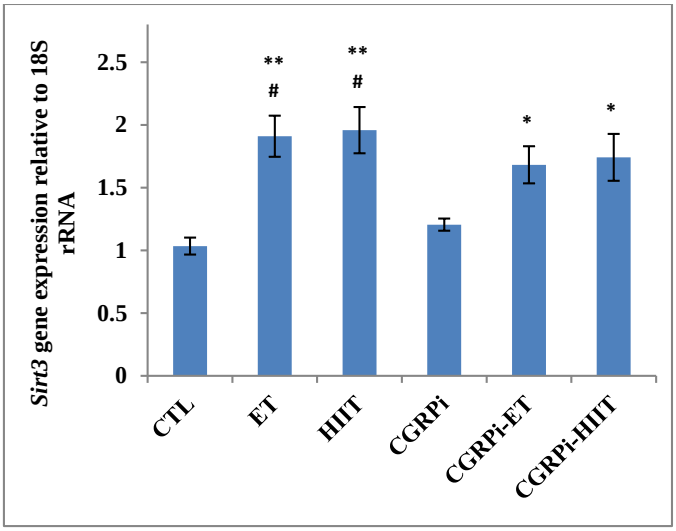


Figure 8. Levels of *Sirt3* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

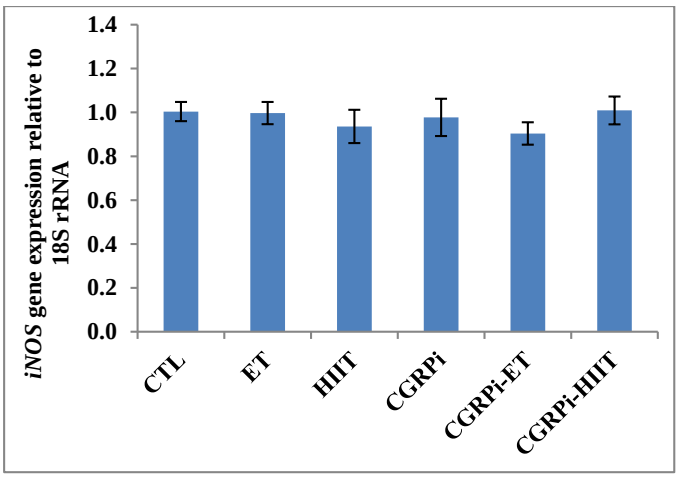


Figure 9. Levels of *iNOS* gene quantified by real-time PCR across six groups: control (CTL), endurance training (ET), high-intensity interval training (HIIT), CGRP inhibitor Olcegepant (CGRPi), CGRPi-ET, and CGRPi-HIIT. Data are presented as Mean ± SEM, with a significance threshold set at $p < 0.05$. Statistical significance is indicated as follows: * denotes a significant difference compared to the control group, # denotes a significant difference compared to CGRPi, with * or # indicating $p < 0.05$, ** or ## indicating $p < 0.01$, and *** or ### indicating $p < 0.001$.

DISCUSSION

Exercise training has profound effects on the cardiovascular system, metabolic flexibility, and mitochondrial dynamics at the molecular level [21, 29]. As a vasodilator, CGRP is intricately linked to migraine pathophysiology, and its antagonists have emerged as promising therapeutic targets in migraine management [31,32]. This study evaluated the effects of ET and HIIT, both individually and in combination with a CGRP antagonist, on aortic gene expression. We found that exercise significantly altered the expression profiles of the targeted genes, markedly increasing eNOS protein, and mRNA levels of *eNOS*, *Pgc-1α*, *Bcl-2*, *Nrf-1*, *Sirt3*, and *Parkin* in exercised rats compared to controls. In contrast, treatment with the CGRP antagonist did not markedly influence gene expression compared to controls; however, the combination of exercise and CGRPi administration resulted in increases of some genes that warrant further investigation.

CGRP receptors can be found in the cardiovascular system, including the heart and blood vessels. CGRP serves as a powerful vasodilator and is crucial for regulating vascular resistance and blood flow to various organs, both under normal physiological conditions and during pathological situations such as cerebral or cardiac ischemia [33]. CGRP signals via 3',5'-cyclic adenosine monophosphate to mediate vasodilation directly in vascular smooth muscle cells and via a NO-dependent vascular relaxation in endothelial cells.[34]

Mitochondria, as dynamic organelles, generate ROS during electron transport. Biogenesis and mitophagy are distinct physiological responses that regulate mitochondrial quantity. Fusion and fission are also associated with changes in mitochondrial size and number. Several factors regulate these processes, including PGC-1α, NRF-1, SIRT3, Parkin, PINK, and mitochondrial transcription factor A.[35-37] This study demonstrated that both ET and HIIT increased the expression of Pgc-1α, Nrf-1, Sirt3, and Parkin genes.

Dysfunctional mitochondria can lead to energy deficits in the brain, predisposing individuals to migraines.[8] NO functions as an endogenous signaling molecule with significant roles in vasculature and mitochondrial biology.[35,38] Elevated oxidative stress and NO levels have been documented during migraine attacks [38]. The increased expression of mitochondrial dynamics-related genes (Pgc-1α, Nrf-1, Sirt3, and Parkin) is anticipated to enhance mitochondrial functionality. Our results indicate elevated eNOS gene and protein expressions in the aorta following ET or HIIT, emphasizing its beneficial NO-mediated cardiovascular role.

It was reported that exercise training increases mitochondrial biogenesis via PGC-1α [13], which also regulates Nrf-1 and Nrf-2 expression [17]. Additionally, eNOS upregulates both PGC-1α and Nrf-1, thereby stimulating mitochondrial biogenesis.[38,39] SIRT3 is an important target of PGC-1α for neutralizing mitochondrial ROS 11. SIRT3 levels rise following exercise in both humans and rodent models [19]. PGC-1α and its downstream factors are very important for enhancing locomotor capacity and mitochondrial efficiency.

Interestingly, administration of CGRPi did not significantly affect gene expression levels. While short-term exercise does not alter CGRP levels, long-term exercise has been shown to enhance CGRP secretion from cardiac tissue 14. Given CGRP's capacity to increase eNOS expression and subsequent NO levels, and the fact that the enzyme eNOS increases the expression of Pgc-1α and Nrf-1,[31,36] changes in these genes in the group that only received CGRPi was expected; however, CGRPi showed no effect on these genes. It is reasonable to conclude that CGRPi does not significantly affect the vasculature at baseline. Furthermore, CGRPi did not increase blood pressure in rats with chronic hypertension, indicating that basal levels of CGRP are important for maintaining cardiac function in this state.[20]

In contrast, the two groups receiving CGRPi and performing ET or HIIT exhibited changes in target gene expression. It has been previously mentioned that prolonged exercise increases CGRP secretion in the heart,[14] with CGRP in endothelial cells inducing NO-dependent vasodilation.[16] Intravenous administration of CGRP has been shown to cause vasodilation and reduce blood pressure in humans. The substantial effects of CGRP on cardiovascular function suggest an important role in the context of cardiovascular disease. In CGRP knockout mice, blood pressure was unaffected by CGRP deletion, while administration of angiotensin II reduced eNOS in these mice.[22] The relationship between prolonged exercise and CGRP secretion suggests potential competition between exercise-induced CGRP elevation and the inhibitory action of CGRP antagonism, necessitating further investigation.

Our findings reveal that CGRP antagonist administration, combined with either ET or HIIT, elevated eNOS expression (gene and protein) along with *Pgc-1α*, *Parkin* and *Nrf-1* gene expression compared to controls and the CGRPi-only group. A

combination of exercise training and CGRPi appeared to increase eNOS expression, and given that exercise increases CGRP secretion,[14] it is suggesting a complex interplay between exercise and CGRP signaling pathways which requires further investigation.

While *Pgc-1α* gene expression during exercise remained unaffected by CGRPi, a slight reduction in expression in the exercise plus CGRPi groups (though not significant) raises considerations for CGRP's indirect modulation on mitochondrial dynamics.

In contrast, Bcl-2 expression decreased in the groups received CGRPi alongside exercise, counteracting the exercise-induced increases, implying that exercise alone increases a more favorable anti-apoptotic state than when combined with CGRPi (CGRPi-ET and CGRPi HIIT). Simultaneously, *Bax* expression remained consistent across groups, indicating no induction of apoptosis due to CGRPi, exercise, or their combination. Unlike *Bcl-2*, CGRP antagonism did not influence exercise-induced increases in *Parkin* and *Nrf-1* expressions. Elevated levels of *Nrf-1* further affirm the direct effect of *Pgc-1α* on regulating its expression.[17]

Sirt3 expression increased significantly among the exercise groups (ET, HIIT, CGRPi-ET, and CGRPi-HIIT) compared to the controls, yet only the ET group exhibited a significant increase concerning the CGRPi group. This pattern indicates that exercise has a greater effect than CGRPi, with minimal alterations in the CGRPi group due to differential expression of genes such as *eNOS*, *Pgc-1α*, *Nrf-1*, and *Parkin*.

Some limitations of our study include the lack of evaluation of circulating CGRP levels, as we focused solely on antagonism its activity. Additionally, CGRPi may affect the amylin receptor, albeit with a lower affinity (approximately 200-fold weaker than the CGRP receptor), which warrants consideration in future studies.[40] Other limitation is about the second-generation CGRP antagonists such as ubrogepant and rimegepant, which have been shown to have improved pharmacokinetics and receptor selectivity. We used one of the first-generation CGRP inhibitor, BIBN 4096, to assess its specific effects. Future investigations will certainly benefit from utilizing second-generation CGRP inhibitors to explore their effects on the cardiovascular system and mitochondrial dynamics. While our study provides valuable insights into the effects of a single dose of a CGRP antagonist in conjunction with a six-week exercise training protocol, several limitations should be acknowledged. Firstly, the use of only a single dosing regimen restricts the understanding of how varying doses and durations may affect the efficacy of CGRP antagonism. Also, there was no information about CGRP inhibitor (Olcegepant) reversibility and we were not able to discuss that. Additionally, the six-week duration of exercise may not adequately reflect chronic adaptations in mitochondrial dynamics and gene expression; thus, longer interventions could yield different results. Another limitation is the absence of histological examinations, which would have elucidated structural changes and cellular responses linked to both CGRP antagonism and exercise. Moreover, potential off-target effects of CGRP antagonism warrant further investigation, as interactions with other pathways may influence the outcome.[41] Finally, caution is necessary when extrapolating our findings to human populations due to inherent species differences in physiology and pharmacology, underscoring the need for additional research in human models.

CONCLUSION

Exercise significantly enhances oxidative capacity through increased mitochondrial biogenesis and upregulation of *Pgc-1α*, *Nrf-1*, and *Sirt3*. Despite CGRP's role in vasodilation, our findings suggest that CGRP antagonism does not influence eNOS expression in the aorta nor does it affect eNOS levels in exercise-performing groups. This indicates that CGRP mediates vasodilation via mechanisms independent of eNOS in the aorta. Importantly, CGRPi does not exhibit detrimental effects on genes implicated in mitophagy and mitochondrial biogenesis in aortic tissue. Given the potential therapeutic applications of CGRP antagonists for migraines, hypertension, and heart failure, our results indicate the safety of CGRP antagonist, though further investigation in experimental and clinical settings is warranted. We hope that our findings can serve as a foundation for future research aimed at elucidating these mechanisms in human subjects, ultimately contributing to improved therapeutic strategies for cardiovascular and migraine-related conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES AND NOTES

1. M. Schurks, PM. Rist, ME. Bigal, JE. Buring, RB. Lipton, T. Kurth. Migraine and cardiovascular disease: systematic review and meta-analysis. *BMJ.* 2009, **339**, b3914.
2. S. Sacco, T. Kurth. Migraine and the risk for stroke and cardiovascular disease. *Curr Cardiol Rep.* **2014**, 16, 524
3. T. Kurth, AC. Winter, AH. Eliassen, R. Dushkes, KJ. Mukamal, EB. Rimm, WC. Willett, JE. Manson, KM. Rexrode. Migraine and risk of cardiovascular disease in women: prospective cohort study. *BMJ.* **2016**, 353, i2610.
4. A. Masuda, K. Shimamoto, Y. Mori, M. Nakagawa, N. Ura, O. Iimura. Plasma calcitonin gene-related peptide levels in patients with various hypertensive diseases. *J Hypertens.* **1992**, 10, 1499-504.
5. HM. Luo, X. Wu, X. Xian, LY. Wang, LY. Zhu, HY. Sun, L. Yang, WX Liu. Calcitonin gene-related peptide inhibits angiotensin II-induced NADPH oxidase-dependent ROS via the Src/STAT3 signalling pathway. *J. Cell Mol Med.* **2020**, 24, 6426-37.
6. A. Dieterle, MJ. Fischer, AS. Link, WL. Neuhuber, K. Messlinger. Increase in CGRP- and nNOS-immunoreactive neurons in the rat trigeminal ganglion after infusion of an NO donor. *Cephalalgia.* **2011**, 31, 31-42.
7. EC. Gross, N. Putananickal, AL. Orsini, DR. Vogt, SP. Sandor, J. Schoenen, D. Fischer. Mitochondrial function and oxidative stress markers in higher-frequency episodic migraine. *Sci Rep.* **2021**, 11, 4543.
8. M. Fila, E. Pawlowska, J. Blasiak. Mitochondria in migraine pathophysiology - does epigenetics play a role? *Arch Med Sci.* **2019**, 15, 944-56.
9. TJ. LaRocca, CM. Hearon, GD. Henson, DR. Seals. Mitochondrial quality control and age-associated arterial stiffening. *Exp Gerontol.* **2014**, 58, 78-82.

10. SM. Craige, S Kroller-Schon, C. Li, S. Kant, S. Cai, K. Chen, MM. Contractor, Y. Pei, E. Schulz, JF. Keaney. PGC-1alpha dictates endothelial function through regulation of eNOS expression. *Sci Rep.* **2016**, 6, 38210.
11. S. Rius-Perez, I. Torres-Cuevas, I. Millan, AL. Ortega, S. Perez. PGC-1alpha, Inflammation, and Oxidative Stress: An Integrative View in Metabolism. *Oxid Med Cell Longev.* **2020**, 2020, 1452696.
12. JC. Ray, M. Kapoor, RJ. Stark, SJ. Wang, L. Bendtsen, M. Matharu, EJ. Hutton. Calcitonin gene related peptide in migraine: current therapeutics, future implications and potential off-target effects. *J. Neurol Neurosurg Psychiatry.* **2021**, 92, 1325-34.
13. Jl. Steiner, EA. Murphy, JL. McClellan, MD. Carmichael, JM. Davis. Exercise training increases mitochondrial biogenesis in the brain. *J. Appl Physiol (1985).* **2011**, 111, 1066-71.
14. XJ. Sun, SS. Pan. Role of calcitonin gene-related peptide in cardioprotection of short-term and long-term exercise preconditioning. *J. Cardiovasc Pharmacol.* **2014**, 64, 53-9.
15. A. Parnow, R. Gharakhanlou, Z. Gorginkaraji, S. Rajabi, R. Eslami, M. Hedayati, R. Mahdian. Effects of endurance and resistance training on calcitonin gene-related Peptide and acetylcholine receptor at slow and fast twitch skeletal muscles and sciatic nerve in male wistar rats. *Int. J. Pept.* **2012**, 2012, 962651.
16. PS. Gaete, MA. Lillo, M. Puebla, I. Poblete, XF. Figueroa. CGRP signalling inhibits NO production through pannexin-1 channel activation in endothelial cells. *Sci Rep.* **2019**, 9, 7932.
17. AP. Gureev, EA. Shaforostova, VN. opov. Regulation of Mitochondrial Biogenesis as a Way for Active Longevity: Interaction Between the Nrf2 and PGC-1alpha Signaling Pathways. *Front Genet.* **2019**, 10, 435.
18. OM. Palacios, JJ. Carmona, S. Michan, Ky Chen, Y. Manabe, JL. Ward, LJ, Goodyear, Q. Tong. Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging (Albany NY).* **2009**, 1, 771-83.
19. BA. Edgett, MC. Hughes, JB. Matusiak, CG. Perry, CA. Simpson, BJ. Gurd. SIRT3 gene expression but not SIRT3 subcellular localization is altered in response to fasting and exercise in human skeletal muscle. *Exp Physiol.* **2016**, 101, 1101-13.
20. T. Skaria, J. Vogel. The Neuropeptide alpha-Calcitonin Gene-Related Peptide as the Mediator of Beneficial Effects of Exercise in the Cardiovascular System. *Front Physiol.* **2022**, 13, 825992.
21. A. Aracil-Marco, JM. Sarabia, D. Pastor, S. Guillen, R. Lopez-Grueso, J. Gallar, M. Moya-Ramon. Acute Increase in Blood alphaCGRP at Maximal Exercise and Its Association to Cardiorespiratory Fitness, Carbohydrate Oxidation and Work Performed: An Exploratory Study in Young Men. *Biology (Basel).* **2021**, 10**.**
22. Z. Kee, X. Kodji, SD. Brain. The Role of Calcitonin Gene Related Peptide (CGRP) in Neurogenic Vasodilation and Its Cardioprotective Effects. *Front Physiol.* **2018**, 9, 1249.
23. A. MaassenVanDenBrink, J. Meijer, CM. Villalon, MD. Ferrari. Wiping Out CGRP: Potential Cardiovascular Risks. *Trends Pharmacol Sci.* **2016**, 37, 779-88.
24. S. Ghatta, D. Nimmagadda. Calcitonin gene-related peptide: Understanding its role. *Indian journal of pharmacology.* **2004**, 36, 277.
25. FA. Pinho-Ribeiro, B. Baddal, R. Haarsma, M. O'Seaghdha, NJ. Yang, KJ. Blake, M. Portley, WA. Verri , JB. Dale, MR. Wessels, IM. Chiu. Blocking Neuronal Signaling to Immune Cells Treats Streptococcal Invasive Infection. *Cell.* **2018**, 173, 1083-97.
26. TR. Glowka, A. Steinebach, K. Stein, T. Schwandt, M. Lysson, B. Holzmann, K. Tsujikawa, WJ. de Jonge, JC. Kalff, S. Wehner. The novel CGRP receptor antagonist BIBN4096BS alleviates a postoperative intestinal inflammation and prevents postoperative ileus. *Neurogastroenterol Motil.* **2015**, 27, 1038-49.
27. S. Lonbro, JM. Wiggins, T. Wittenborn, PB. Elming, L. Rice, C. Pampo, JA. Lee, DW. Siemann, MR. Horsman. Reliability of blood lactate as a measure of exercise intensity in different strains of mice during forced treadmill running. *PLoS One.* **2019**, 14, e0215584.
28. M. Mansouri, R. Nikooie, A. Keshtkar, B. Larijani, K. Omidfar. Effect of endurance training on retinol-binding protein 4 gene expression and its protein level in adipose tissue and the liver in diabetic rats induced by a high-fat diet and streptozotocin. *J Diabetes Investig.* **2014**, 5, 484-91.
29. A. Constans, C. Pin-Barre, F. Molinari, JJ. Temprado, T. Brioche, C. Pellegrino, J. Laurz. High-intensity interval training is superior to moderate intensity training on aerobic capacity in rats: Impact on hippocampal plasticity markers. *Behav Brain Res.* **2021**, 398, 112977.
30. A. Mohammadi, H. Fallah, B. Shahouzehi, H. Najafipour. miR-33 inhibition attenuates the effect of liver X receptor agonist T0901317 on expression of liver X receptor alpha in mice liver. ARYA Atheroscler. 2017, 13, 257-63.
31. VH. Avilés-Rosas, E. Rivera-Mancilla, BA. Marichal-Cancino, G. Manrique-Maldonado, AH. Altamirano-Espinoza, A. Maassen Van Den Brink, CM. Villalón. Olcegepant blocks neurogenic and non-neurogenic CGRPergic vasodepressor responses and facilitates noradrenergic vasopressor responses in pithed rats. *Br J Pharmacol.* **2017**,174, 2001-2014.
32. S. Benemei, P. Nicoletti, JG. Capone, P. Geppetti. CGRP receptors in the control of pain and inflammation Author links open overlay panel. *Current Opinion in Pharmacology.* **2009**,9, 9-14
33. V. Favon, L. Giani, L. Al-Hassany, GM. Asioli, C. Butera, I. de Boer, M. Guglielmetti, C. Koniari, T. Mavridis, M. Vaikjärv, I. Verhagen, A. Verzina, B. Zick, P. Martelletti, S. Sacco. European Headache Federation School of Advanced Studies (EHF-SAS). CGRP and migraine from a cardiovascular point of view: what do we expect from blocking CGRP. *J Headache Pain.* **2019**, 20(1), 27.
34. F. Argunhan, D. Thapa, AA. Aubdool, E. Carlini, K. Arkless, ER. Hendrikse, J. de Sousa Valen‧, X. Kodji, B. Barrett, CA. Ricciardi, L. Gnudi, DL. Hay, SD. Brain. Calcitonin Gene-Related Peptide Protects Against Cardiovascular Dysfunction Independently of Nitric Oxide In Vivo. *Hypertension.* **2021**, 77(4), 1178-1190.
35. E. Nisoli, MO. Carruba. Nitric oxide and mitochondrial biogenesis. J. Cell Sci. 2006, 119, 2855-62.
36. A. Ansari, MS. Rahman, SK. Saha, FK. Saikot, A. Deep, KH. Kim. Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. *Aging Cell.* **2017**, 16, 4-16.
37. K. Palikaras, E. Lionaki, N. Tavernarakis. Balancing mitochondrial biogenesis and mitophagy to maintain energy metabolism homeostasis. *Cell Death Differ.* **2015**, 22, 1399-401.
38. HB. Suliman, CA. Piantadosi. Mitochondrial biogenesis: regulation by endogenous gases during inflammation and organ stress. *Curr Pharm Des.* **2014**, 20, 5653-62.
39. L. Litvinova, DN. Atochin, N. Fattakhov, M. Vasilenko, P. Zatolokin, E. Kirienkova. Nitric oxide and mitochondria in metabolic syndrome. *Front Physiol.* **2015**, 6, 20.
40. CS. Walker, DL. Hay. CGRP in the trigeminovascular system: a role for CGRP, adrenomedullin and amylin receptors? *Br J Pharmacol.* **2013**, 170, 1293-307.
41. P. Bhuee, A. Birdi. Correlation of cardiac markers with thyroid stimulating hormone in subclinical hypothyroid elderly. *Biomed. Ther. Lett.* **2024**, 11 (1), 906.