www.jmscr.igmpublication.org Impact Factor 3.79

ISSN (e)-2347-176x

Journal Of Medical Science And Clinical Research
IGM Publication An Official Publication Of IGM Publication

# Melatonin Reduces Cardiac Injury Induced by Lipopolysaccharides in Rats

Authors

**Ola M. Tork[1], Shaimaa N. Amin[1], Laila A.Rashed[2]**

[1]MD, Physiology Department, Faculty of Medicine, Cairo University
[2]MD, Biochemistry Department, Faculty of Medicine, Cairo University

Corresponding Author
**Shaimaa N. Amin**
Department of Medical Physiology-Faculty of Medicine-Cairo University, 11451
Email: *shaimaa599@yahoo.com*

**ABSTRACT**

*Lipopolysaccharide (LPS) is known to cause inflammation and switch myocardial fuel similar to that of the failing heart: reduced mitochondrial substrate flux and myocyte lipid accumulation. The nuclear receptor: peroxisome proliferator activated receptor-alpha (PPARα) and PPAR-gamma coactivator-1alpha (PGC-1α) play important roles in transcriptional regulation of myocardial metabolism and may contribute to the changes that accompanied cardiomyopathic alterations in chronic inflammation. Melatonin is claimed to have anti-inflammatory activity in animal models. Our work was designed to assess the modulatory effect of melatonin on LPS-induced lipotoxic effect in heart of male albino rats for 6weeks. A systemic vehicle or LPS alone or combined with melatonin was administered. Serum cardiac enzymes, cardiac lipids and 3-nitrotyrosin (3-NT) and the gene expression of cardiac nuclear factor-kappa B (NF-κB), PPARα and PGC-1α were measured. Melatonin treatment showed a significant improvement of cardiac enzymes together with reduced cardiac lipid accumulation, 3-NT and NF-κβ and PPARα that paralleled enhancement of PGC-1α gene expression compared with the LPS rats.We conclude that cardioprotection of melatonin in chronic inflammatory condition is related to restoration of PGC-1α expression in addition to its antioxidant and anti-inflammatory capacity.*

**Keywords:** *Lipopolysaccharide, melatonin, 3-nitrotyrosin; peroxisome proliferator–activated receptor-γ coactivator-1α ; peroxisome proliferator activated receptor-alpha*

## INTRODUCTION

To date, the links between inflammatory signaling and myocardial fuel and energy metabolism remain unclear. Altered heart metabolism was considered a byproduct of heart failure. However, emerging evidence, including observations of the phenotypic expression of genetic defects in humans and animals, supports the notion that derangements in mitochondrial energy metabolism contribute to cardiac dysfunction (Finck and Kelly, 2007)

Peroxisome proliferator activated receptor gamma coactivator-1 (PGC-1) proteins enhance the transcriptional activity of transcription factors through direct protein-protein interactions. Unlike most known transcriptional coactivators; PGC-1α and PGC-1β expression are enriched in tissues with high capacity mitochondrial systems (Puigserver*et al*., 1998). The expression of PGC-1α is induced by conditions known to increase the demand for ATP production such as cold, exercise, and fasting (Baar*et al*., 2002).

Because PGC-1 proteins cannot bind DNA directly, they exert their effects through interactions with transcription factors bound to specific DNA elements in the promoter region of genes. DNA bound transcription factors interact with complexes of coactivator and corepressor proteins (Roeder, 2005). PGC-1α functions as an adaptor or scaffold to recruit other coactivator proteins that remodel chromatin. PGC-1α also docks with a protein called ménage-à-trois 1, which is a component of the cyclin-dependent kinase 7 complex that phosphorylates RNA polymerase II and selectively modulates its activity (Sano *et al*.,2005). PGC-1 interacts with many members of transcription factors to control of diverse cellular energy metabolic pathways (Finck and Kelly, 2006).

The first cardiac PGC-1α target identified was peroxisome proliferator–activated receptor-α (PPARα) (Vega *et al*., 2000). PPAR*α* is a ligand-activated transcription factor involved in the regulation of inflammation, immunity, nutrient metabolism and energy homeostasis (Rakhshandehroo *et al*., 2010).

High expression levels of PPAR*α* are found in organs with elevated rate of fatty acid catabolism (Marx *et al*.,2002 and Bookout*et al*.,2006) Evidence suggests that mice and humans share similar PPAR*α* tissue expression profiles ( Bookout*et al*.,2006) . Natural ligands of PPAR*α* include a variety of fatty acids as well as numerous fatty acid derivatives (Khan and Vanden 2003).

One ubiquitous transcription factor of particular importance in immune and inflammatory responses is nuclear factor-kappa B (NF*κ*B). NF-*κ*B considered as an amplifying mechanism that can exaggerate the disease-specific inflammatory process through the coordinated activation of several inflammatory genes (Barnes and Karin 1997; Pandzic, 2010).

Specifically, it has been shown that activated PPAR*α* binds to c-Jun and to the p65 subunit of NF-*κ*B, thereby inhibiting NF-*κ*B- mediated signaling. Additionally, PPAR*α* induces the inhibitory protein I*κ*B*α*, which normally retains

NF-κB in a nonactive form (Rakhshandehroo*et al*., 2010).

PPARα also governs inflammation by downregulating gene expression via a mechanism called transrepression. The first clue towards anti-inflammatory effects of PPARα came from the observation that PPARα−/− mice exhibit a prolonged inflammatory response in the ear swelling test (Devchand*et al*., 1996).

The major biological role of the PPAR/PGC-1α complex in the myocardium appears to be the transcriptional control of enzymes involved in fatty acid uptake and oxidation (Finck and Kelly, 2007).

PGC-1α activates mitochondrial biogenesis (Baar*et al*., 2002) and induces the expression of reactive oxygen species (ROS) scavengers (St-Pierre *et al*., 2006).

Melatonin (N-acetyl-5-methoxytryptamine) is secreted by the pineal gland of all mammals, including man. A quite number of different organs have the capability to synthesize melatonin (Pandi-Perumal*et al*., 2013). Melatonin participates in diverse functions of the body including sleep and circadian rhythm regulation and immunoregulation (Pandi-Perumal*et al*., 2006). Melatonin seems to have cardioprotective properties via its direct free radical scavenger and its indirect antioxidant activity (Galano*et al*., 2011).

Increased expression and activity of inducible isoform of nitric oxide synthase (iNOS) was demonstrated in the myocardium of animals and patients with heart failure (HF) (Dai *et al*., 2004). The combination of superoxide and NO yields peroxynitrite that has been demonstrated in various HF both in animals and human subjects (Turk and Murad, 2002).

Peroxynitrite is known to inhibit mitochondrial respiration, contractile function of cardiac myocytes (Xie and Wolin, 1996). Therefore, the generation of peroxynitrite by the septic heart may contribute to its dysfunction (Khadour*et al*., 2002) .Nitrotyrosine is a useful estimate of tissue peroxynitrite formation (Xie and Wolin, 1996 ).

This study was designed to investigate the link between LPS- induced overexpression of inflammatory mediators in the lipotoxic induced cardiac injury and the effect of early melatonin treatment.

## MATERIALS AND METHODS

The study protocol was approved by ethical and scientific committee of Physiology Department, Kasr Al Ainy- Faculty of Medicine.

24 adult male albino rats initially weighing 150-200g were used in this study. The rats were obtained from the Animal House Unit of Kasr Al-Aini- Faculty of Medicine, Cairo University. They were housed in cages (4/cage). They were kept at room temperature and normal dark-light cycles with free access to laboratory rat chow and tap water throughout the study.

***Animal Groups (8 rats/group)***

**Group I:**control treated with vehicle.

**Group II**; (LPS group): received LPS (Sigma, st. Louis, MO, USA) 80µg/100g i.p.once (Khairallah*et al*., 2014).

**Group III:** melatonin +LPS co-treatment group, melatonin (Sigma, st. Louis, MO, USA), 10 mg/kg i.p. (Mungrue*et al*.,2002) at 17:00 hr. (Prunet*et al*.,2003) for 6 weeks - 5days / week) started just after the single LPS injection (80µg/100g i.p.). The increased effect of melatonin administration before the end of the light period related to increase density of melatonin receptor (Vanecek, 1998).

At the end of the experimental period, blood samples were taken for the determination of serum cardiac enzymes. The rats were sacrificed and hearts were excised, rapidly frozen at -80°C, for the determination of the level of cardiac lipids , 3-NT , and the gene expression of NFκB , PPAR α and PGC-1α transcription factors.

**Biochemical Measurements**

**Measurement of serum lactate dehydrogenase (LDH) &creatinine phosphokinase (CPK):**

CPK and LDH were detected according to (Rosalki, 1967).

**Detection of Cardiac Lipids**

Part of the heart was homogenized and extracted with isopropanol (1 ml/50 mg) (Oakes *etal.*, 2001). After centrifugation, 5ul aliquots of supernatant were added to 300 ul of reagent for enzymatic colorimetric determination of total cholesterol and triglyceride contents using commercial available kit.

**Detection of 3-NT**

It was detected using ELISA kit supplied by Abcam, USA according to manufacturer's instruction.

**Detection of NFKβ, PPAR α and PGC-1α gene expression using quantitative real time Polymerase chain reaction(qRT–PCR)**

**RNA Isolation and Reverse Transcription**

RNA was extracted from the heart tissue homogenate using the RNeasy plus mini kit (Qiagen, Venlo, TheNetherlands), according to the manufacturer's instructions. Genomic DNA was eliminated by a DNase-on-column treatment supplied with the kit. The RNA concentration was determined spectrophotometrically at 260 nm using the NanoDrop ND-1000 spectrophotometer (ThermoFisher scientific, Waltham, USA). cDNA synthesis reaction was performed using the Reverse Transcription System (Promega, Leiden, The Netherlands). Total RNA was incubated at 70°C for 10 min to prevent secondary structures. The RNA was supplemented with MgCl2 (25mM), RTase buffer (10X), dNTP mixture (10mM), oligo d(t) primers, RNase inhibitor (20 U) and AMV reverse transcriptase (20 U/µl). This mixture was incubated at 42°C for 1 h .

**qRT–PCR**

It was performed in an optical 96-well plate with an ABI PRISM 7500 fast sequence detection system (Applied Biosystems, Carlsbad, California) and universal cycling conditions(min 95°C, 40 cycles of 15 s at 95°C and 60 s at 60°C).

Each 10 µl reaction contained 5 µl SYBR Green Master Mix (Applied Biosystems), 0.3 µl gene-specific forward and reverse primers (10 µM), 2.5 µl cDNA and 1.9 µl nuclease-free water. The sequences of PCR primer pairs used for each gene are shown in Table 1. Data were analyzed with the ABI Prism sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) genes (Kenneth *et al*., 2001).

**Table 1. Primer sequences used for RT-PCR**

| primer | Sequence |
|---|---|
| NFKB | Forward primer: 5′-GCTTACGGTGGGATTGCATT-3′<br>Reverse primer: 5′-TTATGGTGCCATGGGTGATG-3′ |
| PPAR-α | forward primer 5'- ACTTATCCTGTGGTCCCCGG 3 '<br>reverse primer 5′ CCGACAGAAAGGCACTTGTGA -3' |
| PGC-1α | Forward primer 5′- TGTGCAACTCTCTGGAACTG -3'<br>Reverse primer 5′- TGAGGACTTGCTGAGTGGTG -3' |
| GAPDH | forward: 5′- CTCCCATTCTTCCACCTTTG-3′<br>reverse: 5′- CTTGCTCTCAGTATCCTTGC-3′ |

## Data Treatment and Statistics

The results were analyzed using SPSS computer software package, version 21 (IBM, USA). Data were presented as mean±SD. Comparison of quantitative variables between the studied groups was done using Kruskal Wallis test with Wilcoxon signed rank test according to the result of the Shapiro-Wilk test for normality of distribution. Correlations were calculated by Spearman's test. Results were considered statistically significant at $p \leq 0.05$ (Altman, 2005).

## RESULTS

### As Revealed from Table 2

CPK and LDH serum levels showed significant increase in LPS group ($p<0.05$) compared to control. Melatonin treatment resulted in significant decrease in serum CPK and LDH ($p<0.05$) compared to LPS group.

**Table 2: Biochemical Parameters in the studied groups (n=8 rats /group)**

| | Control group | LPS group | LPS +melatonin | P-value |
|---|---|---|---|---|
| CPK( U/l) | 107.65±12.9 | 203.917±6.9075* | 161.971±16.1622*# | .000 |
| LDH ( U/l) | 70.9±16.5 | 100.35±6.76* | 80.7±9.06# | .002 |
| Cardiac lipids(µg/mg) | 6.01±1.64 | 20.5±4.6 * | 8.82±1.94*# | .001 |
| Nitrotyrosin(nmol/mg) | 1.08±.107 | 16.23±3.42* | 4.68±1.90*# | .000 |
| NFKB | 1.34±.24 | 8.30±1.36* | 3.61±1.9*# | .001 |
| PPAR alpha | .1150±.035 | .9967±.135* | .2914±.242# | .002 |
| PGCalpha | 1.19±.16 | .328±.29* | 1.08±.25 # | .004 |

***Significant compared to control at P-value ≤ 0.05***
***# Significant compared to LPS group at P-value ≤ 0.05***

-Cardiac lipids showed significant increase in LPS group ($p<0.05$) compared to control. Melatonin treatment with LPS caused significant decrease in cardiac lipids ($p<0.05$) compared to LPS group.

-3-NT level showed significant increase ($p<0.05$) in LPS group compared to control. Melatonin treatment with LPS resulted in significant decrease ( $p<0.05$) compared to LPS group .

NFκB expression in cardiac tissue of LPS group showed significant increase ($p<0.05$) compared to control. Melatonin treatment with LPS caused significant decrease in NFκB expression ($p<0.05$) compared to LPS group.

-PPARα expression showed significant increase ($p<0.05$) compared to control. Melatonin treatment to LPS group resulted in significant decrease ($p<0.05$) compared to LPS group.

-PGC-1α expression in cardiac tissue significantly decreased in LPS group ($p<0.05$) compared to control. Melatonin treatment with LPS caused significant increase in PGC-1α expression ($p<0.05$) compared to LPS group.

Moreover, the current study showed significant negative correlation between cardiac PGC-1α expression and serum CPK, serum LDH, cardiac lipids, cardiac NFκB relative expression and PPARα relative expression (r= -.659, -.591, -.691, -.604, -.765, respectively and P≤ .005 for all).

However, there was significant positive correlation between 3-NT level and serum CPK, serum LDH, cardiac lipids, cardiac NFκB relative expression and cardiac PPAR-α relative expression (r= .848, .804, .823, .859,.782, respectively and P≤ .001 for all). On the other hand, there was significant negative correlation between 3-NT level and PGC-1α expression (r=-.749; P≤ .001).

There was significant positive correlation between PPAR-α gene expression and cardiac lipids (r=.861, P≤ .001).

**DISCUSSION**

In the present work, we analyze the relationship between a chronic infection triggering inflammation, mimicked by LPS injection, and the development of cardiac injury in rats. We have observed that LPS induced cardiac injury as indicated by significant elevation of serum cardiac enzymes: CPK & LDH. This was accompanied with significant elevation of cardiac lipids in LPS rats compared with controls. The occurrence of cardiac injury was probably related to lipid induced toxicity in the heart (Chiu *et al*., 2001). So we can suggest that LPS stimulation suppresses cardiac mitochondrial fatty acid oxidation leading to myocyte lipid accumulation which was in accordance with the finding of (Schilling *et al*., 2011).

Although plasma free fatty acids (FFA) are the primary carbon fuel for the myocardium but, when elevated, can adversely impact the heart. Recent studies showed that elevated plasma FFA is a strong predictor of sudden cardiac death (Jouven*et al.,* 2001), this could be due to deleterious effects on mitochondrial function or activation of $Na^{+}$ or $Ca^{+2}$ channels (Chiu et al., 2005).

The LPS mediated increase in cardiac enzymes and myocardial lipid accumulation were reduced by melatonin treatment. The effect of melatonin on LPS induced changes in lipid metabolism may be due to altered lipid delivery to the heart as consequence of decreased serum lipids or related to improvement of the mitochondrial function that affect the fatty acid oxidation. But the study of (Schilling *et al*., 2011) demonstrating that the reduction in myocardial triglyceride is not a consequence of altered lipid delivery to the heart, rather an effect of increased PGC-1.

In the current study 3-NT showed significant increase in LPS group compared to control. It can be formed both by NOS-dependent" and by myeloperoxidase (MPO) -dependent "process (Ricciardolo*et al*., 2006). In agreement with our

results:*Khadour et al.,(2002)* showed that in LPS treated rats there was increased nitrotyrosine staining.

Also*Eleuteri and coworkers* demonstrated an increased 3-NT plasma level in patients with congestive heart failure (CHF) and suggested a role for MPO and Tumor Necrosis Factor-α (TNFα) in sustaining this increment (Eleuteri*et al.*, 2009). It is to be noted that an increased oxidative/nitrosative stress has been linked to deterioration of heart function in CHF (Colombo *et al*., 2005).

We demonstrated that melatonin treatment with LPS resulted in significant decrease of 3-NTcompared to LPS group (% difference 71.16%). In agreement to our results; Yaman *et al.,(2010)* showed that concomitant administration of melatonin with LPS resulted in decrease in NT level in rat heart.

Melatonin and its metabolites efficiently interact with various reactive oxygen and reactive nitrogen species and also upregulate antioxidant enzymes and downregulate pro-oxidant enzymes. Moreover, melatonin enters all cells and subcellular compartments and crosses morphophysiologic barriers. These findings have implications for the protective effects of melatonin against cardiac diseases induced by oxidative stress (Galano*et al*., 2011).

In the current work LPS stimulation led to sustained induction of myocardial NFκB as indicated by its significant rise in LPS rats compared with the controls.

There is evidence that NF-kB activity is elevated in the CHF (Anker and von Haehling, 2004). In addition (Tavener and Kubes ,2006) showed that TNFα, interleukine-6 (IL-6), and iNOS are induced by LPS and NFκ_B–dependent manner and have all been implicated in sepsis-induced cardiodepression.

As regard the effect of melatonin treatment, we showed that NF-kB significantly decreased in melatonin treated rats compared to the LPS group. Supporting our results the finding of Venerosoet *al*. (2009) who demonstrated that melatonin prevented cardiac inflammation in a rat model of acute exercise; by reducing iNOS expression and blocking the overexpression of inflammatory cytokines possibly through an NFκB-dependent mechanism .

Also the study of *Tamura et al., (2009)* revealed that; the addition of melatonin prevented LPS-induced iNOS expression in cultured rat endothelial cells and aortic rings by a mechanism that is NFκB-dependent through ‘‘reverse remodeling’’ and this may explain our findings.

To determine the mechanism by which LPS alters myocardial metabolism, we initially focused our investigation on myocardial PPARα, given their ability to “boost” the activity of several transcription factors that regulate mitochondrial FAO (Rakhshandehroo et al., 2010).

Interestingly *Burkartet al.,(2007)* provided evidence that cardiac-specific overexpression of PPARα increased FAO, elevated serum TG, left ventricular hypertrophy and systolic dysfunction which is exacerbated by high fat feeding. These

results suggest that long-term PPARα activation can be deleterious to the heart.

Although previous works on sepsis revealed that PPARα expression together with PGC-1 were down regulated in LPS treated rats (Schilling *et al* ., 2011), we found that cardiac PPARα expression was highly induced after 6w from LPS administration compared with the controls. This may suggest that cardiac PPARα expression recovered early after LPS.

Indeed, there was positive correlation between cardiac PPARα gene expression and cardiac lipids, indicating that its activation could cause cardiac lipotoxicity. While lipotoxic effect of over expressed PPARα may be explained by: its stimulatory role on transcription of FA transporters in a mechanism independent on PGC-1 interaction, which results in more lipid accumulation in the heart than their oxidation. Thus the increased PPARα by LPS treatment in our work and its reduction under melatonin supplementation can't alone explain the observed metabolic changes in the heart.

Given the regulatory role of PGC-1 coactivators in maintaining mitochondrial system and regulating genes involved in the cellular uptake and mitochondrial oxidation of FAs via direct co-activation of PPARα (Vega *et al*., 2000; Roeder .2005) ,we assessed the effect of LPS on the myocardial gene expression of the PGC-1α. We found that LPS injection led to a sustained downregulation of PGC-1α.

We can suppose that elevated 3-NTand NF-κB are required for LPS-mediated suppression of PGC-1α gene expression according to (Schilling *et al*., 2011). Moreover, it was found that blocking the LPS-NFκB axisprevented the downregulation of PGC-1 expression by LPS directly or indirectly (Alvarez-Guardia *et al*., 2010).

Surprisingly the expression of PGC-1α, is down-regulated in several models of HF (Aranyet *al*., 2006). This accounts for the "fuel shift" in substrate preference from FA to glucose, steadily reported in the HF (Huss and Kelly, 2006) . Yet, whether a shift towards glucose utilization is a compensatory or maladaptive event remains to be elucidated (Lehman *et al*., 2009).

Probably our work is the first to demonstrate the interrelation between melatonin: the systemic orchestrator of circadian rhythm (Bechtold, 2008) and PGC-1α in the heart. We showed that melatonin treatment induced PGC-1α, which is supposed to reduce LPS-triggered lipid accumulation in the heart and cardiac injury.

The circadian clock has been reported to regulate metabolism and energy homeostasis in peripheral tissues (Bechtold, 2008). This is achieved by mediating the expression and /or activity of certain metabolic enzymes and transport systems (Kohsaka and Bass, 2007). Thus resetting the circadian clock is another intervention that can lead to cardioprotective effect of melatonin.

## CONCLUSION

Data obtained indicate that melatonin protects against heart lipotoxicity associated with chronic inflammation. Impaired production of noxious mediators involved in the inflammatory process

and down-regulation of the NF-κB signal transduction pathway appear to contribute to the beneficial effects of melatonin.

In addition, our findings revealed a major involvement of the PCG-1α–PPARα interaction in the protective role of melatonin against LPS-induced cardiac injury. These results suggest that PGC-1α activation by melatonin may be one of its underlying mechanisms for cardioprotection.

**Declaration of Interest**

There is no conflict of interest

**Funding**

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Author Contributions**

**Ola M. Tork:**wrote the protocol of the work, performed the experimental work, wrote and revised the manuscript.

**Shaimaa N. Amin:** performed statistical analysis, wrote and revised the manuscript.

**LailaA.Rashed:** performed the biochemical analysis.

**ACKNOWLEDGEMENTS**

We would like to thank Mrs. Afaf Mohamed Aify and Mrs. AzaKamal the technicians at Department of Medical Physiology for their kind help in this work.

**REFERENCES**

1. Altman,D 2005 Comparing groups: three or more independent groups of observations in practical statistics for medical research. Chapman and Hall.
2. Alvarez-Guardia D, Palomer X, Coll T, Davidson MM, Chan TO, Feldman AM, Laguna JC, Vazquez-Carrera M. 2010 The p65 subunit of NF-_B binds to PGC-1_, linking inflammation and metabolic disturbances in cardiac cells. *Cardiovasc Res*.;87:445– 458.
3. Anker S T, von Haehling S .2004 INFLAMMATORY MEDIATORS IN CHRONIC HEART FAILURE:AN OVERVIEW. Heart ;90:464–470.
4. Arany Z, Novikov M, Chin S, Ma Y, Rosenzweig A, Spiegelman BM 2006 Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-_ coactivator 1_. ProcNatlAcadSci USA,; 103:10086–10091.
5. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO 2002 Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J*.;16:1879–1886.
6. Barnes PJ & Karin 1997 M:NUCLEAR FACTOR -*k*B — A PIVOTAL TRANSCRIPTION FACTOR IN CHRONIC INFLAMMATORY DISEASES. The New England Journal of Medicine. ; 336:1066-71.

7. Bechtold D 2008 A.Energy-responsive time keeping. *J. Genet* ; 87, 447–458.
8. Bookout A. L., Jeong Y., Downes M., Yu R. T., Evans R. M., and Mangelsdorf D. J.2006 "Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network," *Cell*,, vol. 126, no. 4, pp. 789–799.
9. Burkart EM, Sambandam N, Han X, Gross RW, Courtois M, Gierasch CM, Shoghi K, Welch MJ, Kelly DP 2007 Nuclear receptors PPAR beta/delta and PPAR alpha direct distinct metabolic regulatory programs in the mouse heart. J Clin Invest; 117:3930–3939.
10. Chiu HC, Kovacs A, Blanton RM, Han X, Courtois M, Weinheimer CJ, Yamada KA, Brunet S, Xu H, Nerbonne JM, Welch MJ,et al.2005 Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy. Circ Res ;96:225–233.
11. Chiu HC, Kovacs A, Ford DA, Hsu FF, Garcia R, Herrero P, Saffitz JE, Schaffer JE. 2001 A novel mouse model of lipotoxic cardiomyopathy. J Clin Invest;107: 813–822.
12. Colombo PC, Banchs JE, Celaj S. 2005 Endothelial cell activation in patients with decompensated heart failure. Circulation ;111:58–62.
13. Dai ZK, Tan MS, Chai CY, Yeh JL, Chou SH, Chiu CC, Jeng AY, Chen IJ, Wu JR.2004 Upregulation of endothelial nitric oxide synthase and endothelin-1 in pulmonary hypertension secondary to heart failure in aorta-banded rats. PediatrPulmonol. ;37(3):249–56.
14. Devchand P. R., H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli,1996 "The PPAR$\alpha$-leukotriene B4 pathway to inflammation control," *Nature*, vol. 384, no. 6604,pp. 39–43.
15. Eleuteri E, Di Stefano A, Ricciardolo FL, Magno F, Gnemmi I, Colombo M, Anzalone R, Cappello F, La Rocca G, TarroGenta F et al.2009 Increased nitrotyrosine plasma levels in relation to systemic markers of inflammation and myeloperoxidase in chronic heart failure. Int J Cardiol. 10;135(3):386-90.
16. Finck BN and Kelly DP. 2006 PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest*.;116:615– 622.
17. Finck BN and Kelly DP.2007 Peroxisome Proliferator Activated Receptor γ Coactivator-1 (PGC-1) Regulatory Cascade in Cardiac Physiology and Disease. *Circulation.*; 115:2540-2548
18. Galano A, Tan DX, Reiter RJ. (2011) Melatonin as a natural ally against oxidative stress: a physicochemical examination. J Pineal Res ; 51:1–16.
19. Huss JM, Kelly DP.2005 Mitochondrial energy metabolism in heart failure: a question of balance. J Clin Invest ;115:547–555.

20. Jouven X, Charles MA, Desnos M, Ducimetiere P.2001 Circulating nonesterified fatty acid level as a predictive risk factor for sudden death in the population. Circulation ;104:756–761.
21. Kenneth J. Livak and Thomas D. Schmittgen 2001 Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 22DDCT .METHODS,; 25, 402–408 .
22. Khadour FH, Panas D, Ferdinandy P, Schulze C, Csont T, Lalu MM, Wildhirt SM, Schulz R.Enhanced 2002 NO and superoxide generation in dysfunctional hearts from endotoxemic rats. Am J Physiol Heart Circ Physiol.; 283(3):H1108-15.
23. Khairallah, M. I.; Kassem, L. A.; Yassin, N. A.; Gamal El Din, M. A.; Zekri, M.; Attia, M.2014 The Hematopoietic Growth Factor "Erythropoietin" Enhances the Therapeutic Effect of Mesenchymal Stem Cells in Alzheimer's Disease. Pakistan Journal of Biological Sciences ; 17 ( 1) 9-21. 13p.
24. Khan S. A. and VandenHeuvel J. P.2003 "Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (review)," *Journal of Nutritional Biochemistry*, vol. 14,no. 10, pp. 554–567.
25. Kohsaka A, Bass J.2007 A sense of time: h ow molecular clocks organize metabolism. Trends Endocrinol Metab.; 18:4-11.
26. Lehman J J, Barger P M, Kovacs A, Saffitz J E, Medeiros D M and Kelly D P.2009 Metabolic remodelling of the failing heart: beneficial or detrimental? Cardiovascular Research; 81, 420–428.
27. Marx N1, Kehrle B, Kohlhammer K, Grüb M, Koenig W, Hombach V, Libby P, Plutzky J.2002 "PPAR activators as antiinflammatory mediators in human T lymphocytes: implications for atherosclerosis and transplantation associated arteriosclerosis," *Circulation Research*, vol. 90, no. 6, pp. 703–710.
28. Mungrue IN, Gros R, You X, Pirani A, Azad A, Csont T, Schulz R, Butany J, Stewart DJ, Husain M.2002 Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. J Clin Invest.;109(6):735–43.
29. OAKES, N.D., THALEN, P.G., JACINTO, S.M. & LJUNG, B. 2001 Thiazolidinediones increase plasma-adipose tissue FFA exchange capacity and enhance insulin-mediated control of systemic FFA availability. Diabetes; 50, 1158–1165.
30. Pandi-Perumal S R , BaHammam AS, Brown G.M, Warren Spence D, Bharti VK , Kaur C, Hardeland R, Cardinali DP.2013 Melatonin Antioxidative Defense: Therapeutical Implications for Aging and Neurodegenerative Processes. Neurotox Res ; 23:267–300.

31. Pandi-Perumal SR, Srinivasan V, Maestroni GJM, Cardinali DP, Poeggeler B, Hardeland R 2006 Melatonin: nature's most versatile biological signal? FEBS J ; 273(13):2813–2838.
32. PandzicJaksic V 2010 Adipocytokines as mediators of metabolic role of adipose tissue. *Acta Med Croatica* , 64:253-262.
33. Prunet-Marcassus B, Desbazeille M, Bros A, Louche K, Delagrange P, Renard P, Casteilla L, And Pe´ Nicaud L 2003 Melatonin Reduces Body Weight Gain in Sprague Dawley Rats with Diet-Induced Obesity. Endocrinology ; 144: 5347–5352.
34. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*. ; 92:829–839.
35. Rakhshandehroo M, Knoch B, M¨uller M, and KerstenS :Peroxisome 2010 Proliferator-Activated Receptor Alpha Target Genes. PPAR Research , Article ID 612089, 20 pages
36. Ricciardolo FLM, Di Stefano A, Sabatini F, Folkerts G.2006 Reactive nitrogen species in the respiratory tract. Eur J Pharmacol ;533:240–52.
37. Roeder RG.2005 Transcriptional regulation and the role of diverse coactivators in animal cells. *FEBS Lett*. ;579:909 –915.
38. Rosalki SB.1967 An improved procedure for serum creatine phosphokinase determination. J Lab Clin Med.;69:696–705.
39. Sano M, Izumi Y, Helenius K, Asakura M, Rossi DJ, Xie M, Taffet G, Hu L, Pautler RG, Wilson CR,et al.2007 Menage-a-trois 1 is critical for the transcriptional function of PPARgammacoactivator 1. *Cell Metab*.;5:129 –142.
40. Schilling J, Lai L, Sambandam N, Dey C E,. Leone T C and Kelly D P 2011:Toll-Like Receptor-Mediated Inflammatory Signaling Reprograms Cardiac Energy Metabolism by Repressing Peroxisome Proliferator-Activated Receptor γ□Coactivator-1 signaling. Circ Heart Fail.;4:474-482;
41. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, Handschin C, Zheng K, Lin J, Yang W, et al. 2006 Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell*.;127:397– 408.
42. Tamura EK, Cecon E, Monteiro AW, Silva CL,2009 Markus RP. Melatonin inhibits LPS-induced NO production in rat endothelial cells.J Pineal Res;46:268–274.
43. Tavener SA, Kubes P.2006 Cellular and molecular mechanisms underlying LPS-associated myocyte impairment. *Am J Physiol Heart Circ Physiol*.;290:H800–H806.

44. Turko IV, Murad F.2002 Protein nitration in cardiovascular diseases. Pharmacol Rev;54(4):619–34.
45. Vanecek J .1998 Cellular mechanisms of melatonin action. Physiol Rev ; 78: 687–721.
46. Vega RB, Huss JM and Kelly DP.2000 The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol*. ;20:1868 –1876.
47. Veneroso C, Tuñón MJ, González-Gallego J, Collado PS 2009 Melatonin reduces cardiac inflammatory injury induced by acute exercise. J Pineal Res ;47:184–191
48. Xie YW and Wolin MS.1996 Role of nitric oxide and its interaction with superoxide in the suppression of cardiac muscle mitochondrial respiration. Involvement in response to hypoxia/reoxygenation.Circulation,; 94: 2580–2586.
49. Yaman .H, T. Çayci.T, M. Seyrek.M, Akgül E. Ö. , Y. Gülcan Kurt, İ. Aydin, H. Yaren, E. Çakir, Ö. Özcan, B. Çimen, N. 2010 Effects of vitamin A and C and melatonin on 3-nitrotyrosine formation in guinea pig heart under lipopolysaccharide-induced stress. Turk J Med Sci ; 40 (5): 715-721.