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# Compensative Response Elicited towards Steatosis in High Fat Diet-induced Non-alcoholic Fatty Liver Disease in Rats

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## Abstract

Non-alcoholic fatty liver disease (NAFLD) is the condition in which hepatic fat accumulation is present after exclusion of all other causes of hepatic steatosis such as liver disease caused by other factors, excessive alcohol consumption, and other conditions that may lead to hepatic steatosis. Structural and functional alterations in mitochondria contribute to the pathogenesis of NAFLD. In this study, we investigated the impact of high fat diet on gene expression of SIRT1 and PGC-1 α in experimental non-alcoholic fatty liver disease. The results showed upregulation of both PGC-1 α and SIRT1 gene expression in fatty liver group in comparison to control group. This finding is explained as an adaptive response elicited by the hepatocytes against the high fat diet induced-steatosis.

**Keywords:** Fatty liver; mitochondrial biogenesis; reactive oxygen species; steatosis; steatohepatitis; SIRT1; PGC-1 α ; TNF α

## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the condition in which hepatic fat accumulation is present after exclusion of all other causes of hepatic steatosis such as liver disease caused by other factors, excessive alcohol consumption, and other conditions that may lead to hepatic steatosis. The clinical spectrum of NAFLD is wide-ranging and spans non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). [1]

  

Clinical importance of NAFLD has grown in last few years, mainly in consequence of the obesity epidemics, sedentary lifestyle and high calorie diet adopted by people of developed countries. NAFLD has been considered the most common liver disease and also the most frequent cause of elevated aminotransferases. [2]

Epidemiology of NAFLD worldwide has been evaluated by several reports to allocate healthcare resources and develop national strategies. The most accurate estimation of the global prevalence of NAFLD is 24%-25% of the population. [3] The ascending prevalence of NAFLD is in parallel to the huge spread of obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS). [4] Although several aspects in the pathogenesis of NASH remain unclear, it is now well established that accumulation of excess lipids, especially in the form of free fatty acids, results in toxic damage to the hepatocytes, or lipotoxicity, which triggers inflammation and tissue repair in the form of fibrosis. [5] In this context, the generation of reactive oxygen intermediates has a pivotal role in the induction of damage to the cellular membranes and DNA. [6]

Structural and functional alterations in mitochondria contribute to the pathogenesis of NAFLD. Structural alterations encompass depletion of mitochondrial DNA, morphological and ultrastructural changes, while functional alterations include the respiratory chain and mitochondrial β -oxidation. [7] If mitochondrial or peroxisomal function cannot handle the increased lipid flux, respiratory oxidation may collapse with impairment of fat homeostasis, generation of lipid derived toxic metabolites and overproduction of reactive oxygen species (ROS). [8] All these molecules activate inflammatory pathways contributing to hepatocytes necroinflammation [9] and worsening of mitochondrial damage. [10]

It has been indeed demonstrated that there is a correlation between insulin resistance, obesity, tumor necrosis factor alpha (TNF- α ) levels and mitochondrial dysfunction. [10] Furthermore ROS, together with oxidized LDL particles, may activate Kupffer and hepatic stellate cells, leading to inflammation and fibrosis. [11] Whether mitochondrial dysfunction is a key pathogenic event in NAFLD or the consequence of an altered lipid metabolism still remains unclear. [12]

PGC-1 α is a major regulator of mitochondrial biogenesis. [13] Being a co-transcriptional regulation factor, PGC-1 α induces mitochondrial biogenesis by activating different transcription factors, including NRF-1 and NRF-2, which promote the expression of Tfam. NRF-1 and NRF-2 are important contributors to the sequence of events leading to the increase in transcription of key mitochondrial enzymes, and they have been shown to interact with Tfam, which drives transcription and replication of mtDNA. [14] Being a key regulator of metabolic adaptation, PGC-1 α activity needs to be finely regulated also at a post-transcriptional level. Inhibition by acetylation by, for example, histone acetyltransferase GCN5, and activation by SIRT1 deacetylase represent the major cycle of regulation in response to metabolic changes [15]

In this study, we investigated the impact of high fat diet on gene expression of SIRT1 and PGC1alpha in experimental non-alcoholic fatty liver disease.

## 2. Materials and Methods

The study was conducted on 20 Wistar male rats weighing approximately 100-120 grams obtained from animal house of Medical Research Institute of Alexandria University. Animals were housed 5 per cage with food and water *ad libitum*, on 12:12-h. light-dark cycle at 25 ± 1 ° C. All animals were kept under observation for one week prior to study for acclimation to laboratory environment. Rats were divided equally into the following two groups: group I (control group) received standard rat chew diet (in pellet form) (N=10), group II (the fatty liver group) that included animals received high fat diet (HFD). The HFD consists of commercial rat chew plus peanuts, milk chocolate, and sweet biscuit in a proportion of 3:2:2:1, at the twelfth week fatty liver disease was confirmed by histopathological examination of the liver through scarification of some rats. After induction period, all rats were sacrificed by cervical dislocation to obtain blood samples and liver tissues. Blood was collected from the inferior vena cava, centrifuged, and sera were stored for further analysis. Livers were rapidly dissected out, weighed,

  

snap-frozen in liquid nitrogen and kept at -80℃ for RNA and protein extraction. A portion of the liver was immediately fixed in formalin for histological analyses. All animals received human care, and experimental protocols were conducted according to the principles in the Guide for the Care and Use of Laboratory Animals, and was approved by the Institutional Animal Ethics Committee.

### 2.1.Serum Aminotransferase Levels

Blood samples were centrifuged at 3000 r.p.m. for 10 min at 4° C to obtain serum, that was kept at -80° C until analyzed. Serum alanine-aminotransferase activity was determined using a commercially available kit (DiaSys Diagnostic Systems GmbH, Germany).

### 2.2.Serum lipid profile

Serum cholesterol, serum high-density lipoprotein cholesterol (HDL), serum low-density lipoprotein cholesterol (LDL), and triglycerides were determined on the basis of an enzymatic colorimetric method. [16] using kit purchased from Biosystem Co. Spain.

### 2.3.Hepatic lipid content

To extract lipids 50 mg of liver tissue was homogenized in 5 ml of a chloroform/ methanol (2:1) mixture. The extract was centrifuged at 2500 x g for 15 minutes and supernatant collected and evaporated to dryness under nitrogen. The residue was subsequently reconstituted in a solution of isopropyl alcohol containing 10% tritron X and centrifuged at 10000 x g for 10 minutes. The supernatant was used for the determination of triglycerides and cholesterol content using commercial kits from (Biosystem, Spain) as described previously.

### 2.4 Detection of serum and hepatic tumor necrosis factor alpha

Tumor necrosis factor alpha was detected by sandwich-ELISA as a method [17] using kits purchased from Bioneovan Co. China.

### 2.5 Detection of hepatic sirtuin1

Hepatic SIRT1 were detected by sandwich-ELISA as a method [17] using kits purchased from Bioneovan Co. China.

### 2.6 Liver Histology

A portion of liver tissue was fixed by immersion in 10% buffered formalin (pH 7.4) for 24hr. The fixed tissue was dehydrated in graded ethanol, paraffin-embedded and sectioned at a thickness of 4mm. Hematoxylin-eosin and Sirius Red stainings were performed as previously described, [18] and liver histology was evaluated by an experienced hepato-pathologist.

### 2.7 RNA Isolation and Quantitative PCR

The expression of genes (SIRT1, PGC-1 α and PGDPH (House keeping gene)) was performed using reverse transcriptase polymerase chain reaction (RT-PCR) as previously described [19]. The total were first isolated using appropriate kit and then reverse transcribed using reverse transcriptase enzymes to form complementary DNA (cDNA). The cDNA was then amplified using specific primer sets for each gene by using PCR technique (Table 1). All reagents were purchased from iNtRON Biotechnology, Inc (Foster City, CA, USA). Relative gene expression was calculated as 2-DCt (DCt equal Ct of the target gene minus Ct of GAPDH).

**Table 1: Primer sets (Thermofischer scientific, USA)**

| Gene name | Primer | Primer Sequence |
|---|---|---|
| SIRT1 | Forward | 5-CCAGATTTCAAGGCTGTTGGTTCC-3 |
| | Reverse | 5-CCACAGGAACTAGAGGATAAGGCGT-3 |
| PGC-1α | Forward | 5-TTCAGTGTCACCACCGAAATCCTTAT-3 |
| | Reverse | 5-AGAGGATCTACTGCCTGGGGACC-3 |
| GADPH | Forward | 5-TCACACAATGCAATCCGTTT-3 |
| | Reverse | 5-GGCCTTGACCTTGTTCATGT-3 |

  

### 2.8 Statistical analysis

Data will be expressed as mean ± S.D. Statistical significance of difference between groups will be tabulated and determined by one-way analysis of variance (ANOVA) followed by paired-t test and chi-square test.

## 3. Results

Results showed a significant elevation of both : Serum alanine aminotransferase (ALT) and serum gama-glutamyltranseferase (GGT) activities in fatty liver group compared to control group (P<0.001), on the other hand, there was no significant difference between both groups regarding serum aspartate aminotransferase (AST) activities (Figure 1).

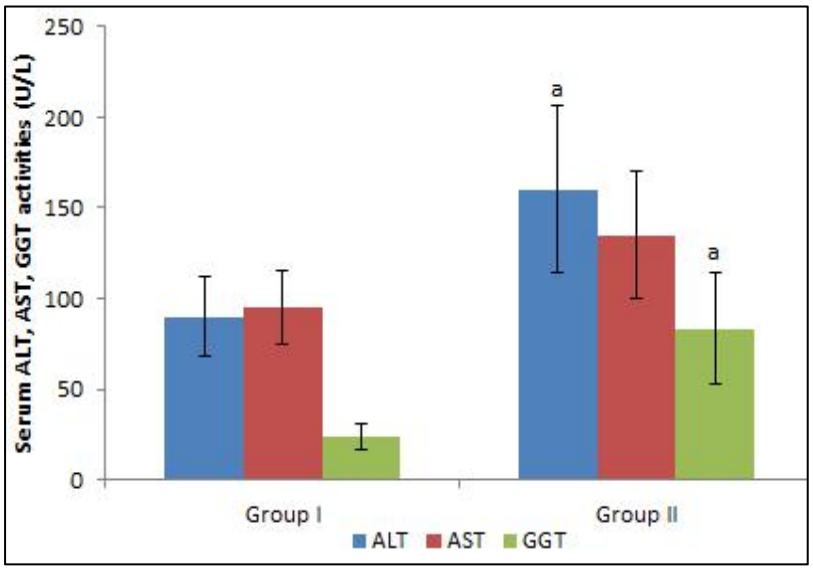


**Figure 1: Serum activities of ALT, AST, GGT in studied groups**

Results also showed a significant elevation of serum levels of cholesterol, LDL and triglycerides in fatty liver group compared to control group ( P<0.001), on the other hand, there no significance difference between fatty liver and control groups concerning serum HDL (Figure 2).

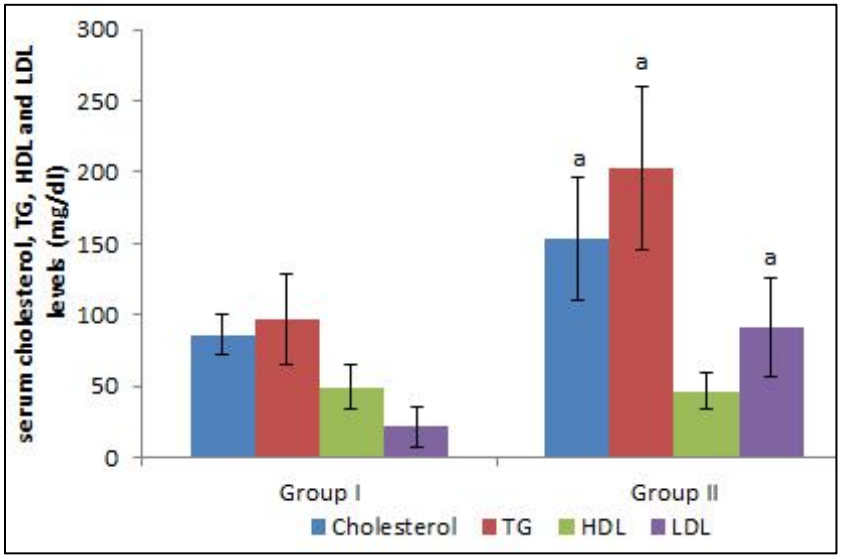


**Figure 2: Serum levels of cholesterol, TG, HDL and LDL**

  

Hepatic cholesterol and triglycerides (TG) contents were significantly elevated in fatty liver group compared to control groups (P<0.001) (Figure 3).

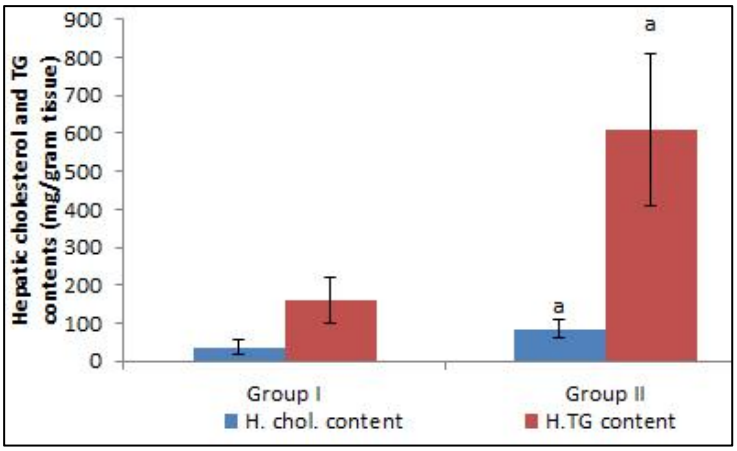


**Figure 3: Hepatic cholesterol and TG contents in studied groups**

Moreover, Histological analysis rat liver sections of animals fed on HFD revealed evidence of marked hepatic degeneration with the presence of micro and macrovesicular steatosis as well as, congested veins and vacuolated cytoplasm were noticed (Figure 4, 5).

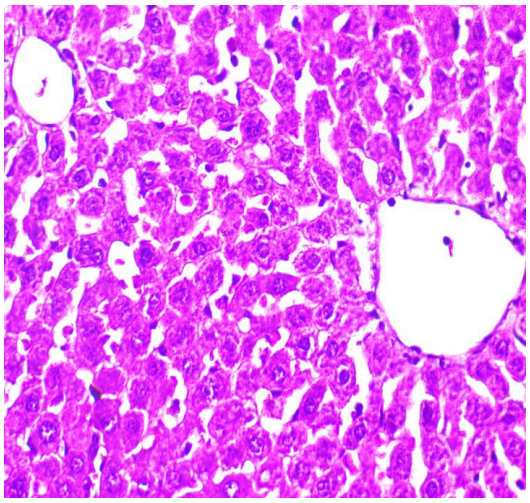

**Figure 4: Control rat liver section showing normal hepatic architecture. (H&E ×400)**

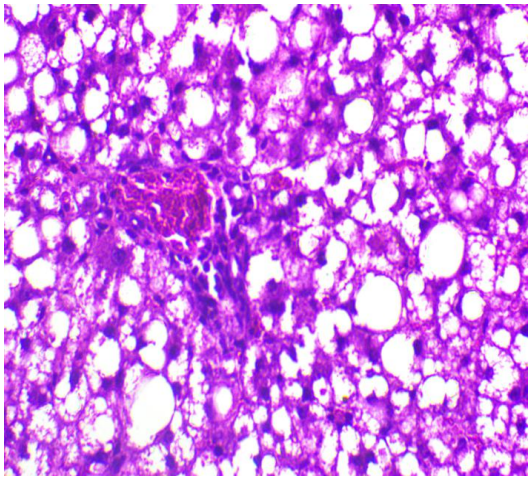

**Figure 5: Rat liver section in NAFL group. (H&E ×400)**

  

Serum levels and hepatic contents of tumor necrosis factor alpha were elevated in fatty liver group compared to control group but insignificant. Hepatic sirtuin1 concentrations were significantly elevated in fatty liver group in comparison to control group (P<0.001) (Figure 6).

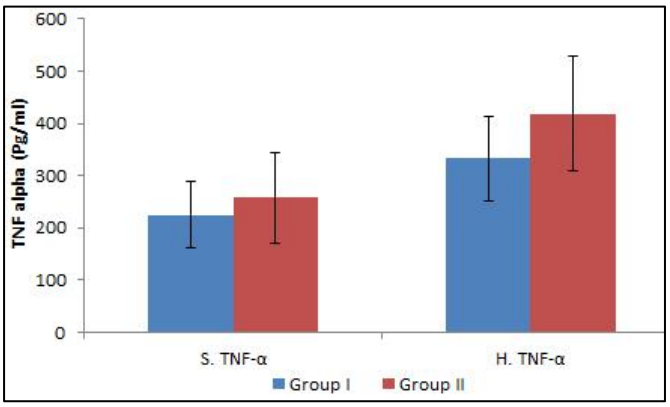


**Figure 6: Hepatic content and serum level of TNFα in both groups**

The current study results also showed upregulation of hepatic PGC-1α expression compared to control group (P<0.001) (Figure 7).

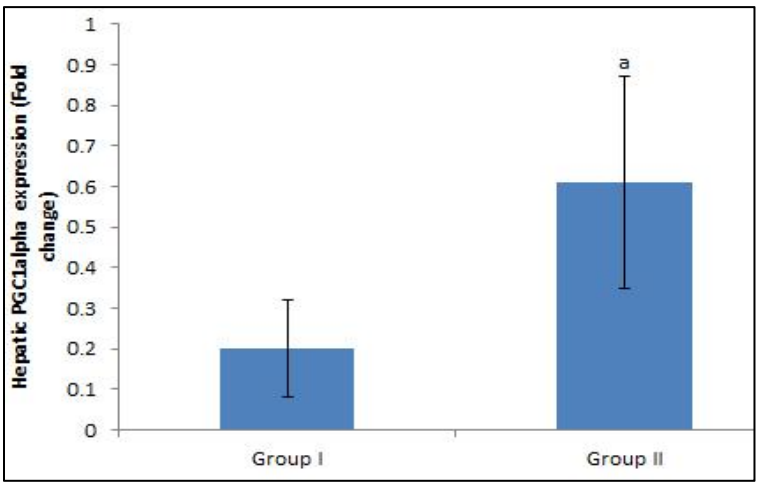


**Figure 7: Hepatic PGC-1α gene expression in both groups**

Moreover, SIRT1 gene expression was upregulated in fatty liver group in comparison to control group (P<0.001) (Figure 8).

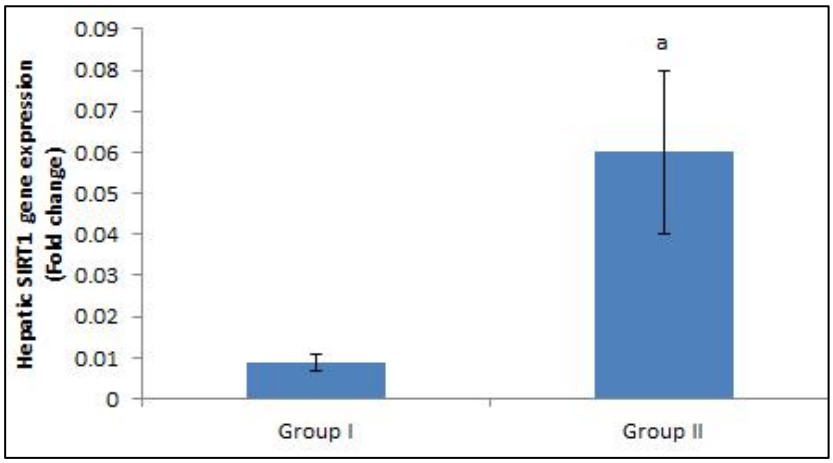


**Figure 8: Hepatic SIRT1 gene expression in both groups**

  

In addition, the results also showed elevated hepatic contents of SIRT1 in fatty liver group compared to control group (P<0.001) (Figure 9).

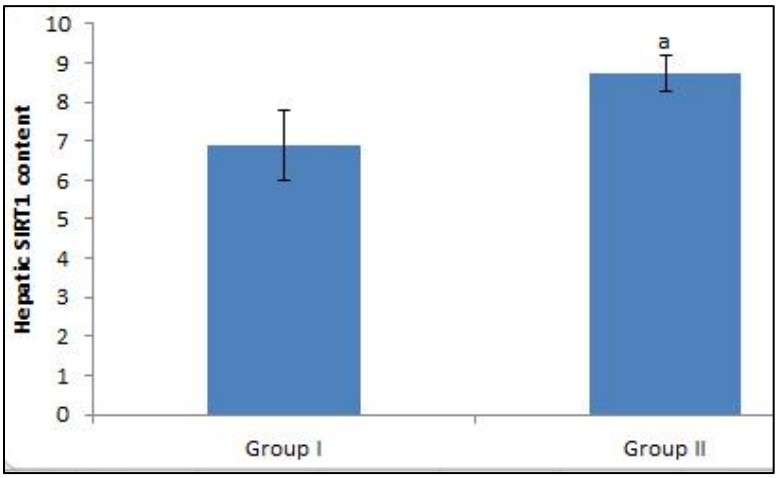


**Figure 9: Hepatic SIRT1 contents in both groups**

## 4. Discussion

The current study revealed the upregulation of SIRT1 and PGC1alpha gene expression in fatty liver group in comparison to control group. Moreover, the levels of SIRT1 protein in untreated group are higher than that of control group. NAFLD might be considered a mitochondrial disease. Mitochondrial dysfunction contribute to the pathogenesis of NAFLD since it affects hepatic lipid homeostasis, promotes ROS production and lipid peroxidation, cytokine release and cell death. [20]

ROS production causes lipid peroxidation of mitochondrial membranes which can contribute to impaired mitochondrial function and perpetuate the ROS generation. Oxidative stress also triggers production of inflammatory cytokines, causing inflammation and fibrogenic response. This ultimately results in the development of NASH. [21] A number of transcription factors and co-regulators are involved in the regulation of cellular and mitochondrial metabolism. The transcriptional co-regulators sense changes in metabolism and regulate gene expression accordingly. One of the most characterized co-activators is PGC-1α. [22]

PGC-1α is a major regulator of mitochondrial biogenesis. [23] Being a co-transcriptional regulation factor, PGC-1α induces mitochondrial biogenesis by activating different transcription factors, including NRF-1 and NRF-2, which promote the expression of Tfam. NRF-1 and NRF-2 are important contributors to the sequence of events leading to the increase in transcription of key mitochondrial enzymes, and they have been shown to interact with Tfam, which drives transcription and replication of mtDNA. [24]

Wa Z et al [25] demonstrated that PGC-1α stimulates mitochondrial biogenesis and respiration in muscle cells through an induction of UCP (uncoupling protein) 2 and through induction of NRF-1 and NRF-2 gene expression. Moreover, PGC-1α binds to and co-activates the transcriptional function of NRF-1 on the promoter for Tfam.

In addition to NRFs, PGC-1α also interacts with and co-activates other transcription factors such as PPARs, thyroid hormone, glucocorticoid, estrogen and estrogen-related receptors α and γ (ERRs). [26] ERRs are orphan nuclear receptors that target vast gene networks involved in all aspects of energy homoeostasis, including fat and glucose metabolism, as well as mitochondrial biogenesis and function. [27] All these previous findings confirm the role of PGC-1α as a major player in mitochondrial biogenesis. The potential of SIRT1 to deacetylate and activate PGC-1α increasing its transcriptional activity may confirm the possible role of SIRT 1 in mitochondrial biogenesis.

In the light of these facts, upregulation of Sirtuin1 and PGC-1α in fatty liver group is possibly explained as an adaptive response against HFD-induced steatosis. In other words, a trial undergone by the cell to compensate against the deleterious

  

effects induced by HFD. These deleterious effects may involve a defected mitochondrial biogenesis in steatotic hepatocytes.

This phenomenon is shared with another study done by Aharoni-Simon MA et al, [28] in which levels of PGC-1α mRNA were elevated in fatty liver group compared to its levels in control group. They stated that the impairment of mitochondrial biogenesis process was found in steatotic livers. However, the increase in PGC-1α mRNA expression, which is most likely an attempt of the hepatocytes to compensate for the decrease in mitochondrial number, did not increase mitochondrial mass through the mitochondrial biogenesis pathway or PGC-1α activity. Prolonged dysfunction of the mitochondrial biogenesis process may lead to increased oxidative stress and modify overall cellular function, which may contribute to progression of NAFLD to NASH.

However, due to the limited number of animals in the present research, further studies are recommended to elucidate the pathogenesis of NAFLD.

## 5. Conclusion

Upregulation of Sirtuin1 and PGC-1α in fatty liver group can be explained as an adaptive response against HFD-induced steatosis, a trial undergone by the cell to compensate against the deleterious effects induced by HFD.

## Abbrevviations

NAFLD: Nonalcoholic fatty liver disease
NAFL: Nonalcoholic fatty liver
MS: metabolic syndrome
NASH: non-alcoholic steatohepatitis
HCC: hepatocellular carcinoma
T2DM: type 2 diabetes mellitus
ROS: reactive oxygen species
TNF-α: tumor necrosis factor alpha
ALT: Serum alanine aminotransferase
AST: serum aspartate aminotransferase
GGT: serum gama-glutamyltranseferase
TG: triglycerides
HDL: serum high-density lipoprotein cholesterol
LDL: serum low-density lipoprotein cholesterol
HFD: high-fat diet

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