The Pre-Conception Maternal Exposure To Sofosbuvir Impacts The Mitochondrial Biogenesis In Prenatal Fetal Tissues: Experimental Study On Rats

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Abstract

Hepatitis C virus (HCV) infection is a global public health problem and Egypt has the highest HCV prevalence worldwide. Hence, global efforts target to eliminate HCV by 2030. Sofosbuvir is a nucleotide analogue inhibitor of HCV polymerase essential for viral replication. Animal studies prove that Sofosbuvir metabolites cross the placenta and are excreted in the milk of nursing animals. We aimed to investigate the possible effects of preconception maternal exposure to Sofosbuvir on the mitochondrial biogenesis in prenatal fetal liver, skeletal muscle and placental tissues using female rats. The study was conducted on 20 female albino rats classified into 2 groups, control group including 10 healthy rats receiving placebo, and exposed group including 10 rats receiving 4 mg/kg of Sofosbuvir. At the end of the 3-month treatment period, pregnancy was induced in both groups by mating with healthy male rats overnight. At gestational day 17, all pregnant female rats were sacrificed. Each fetus was dissected to obtain the fetal liver, skeletal muscle and placental tissues. The results of our study indicated that the preconception exposure of young female rats to Sofosbuvir affects pregnancy outcomes, decreases fertility, and impacts mitochondrial biogenesis and functions in prenatal fetal liver, skeletal muscle and placental.

Highlights

- Sofosbuvir may directly or indirectly affect the ova through suppressing expression of PGC-1α.
- Also suppressing expression of Tfam that drives fetal abnormalities developed later after pregnancy,
- Sofosbuvir may induce placental abnormalities and oxidative stress early in pregnancy
- This affects the nutrient supply to the fetus which results in increased risk of mtDNA mutations.
- Sofosbuvir may affect the hormonal homeostasis preconception and during pregnancy that may affect the fetal metabolism.

1. Introduction

Hepatitis C virus (HCV) infection is a major public health problem worldwide with an estimated global prevalence of 1% that accounts for more than 71 million infections [1], and a high mortality rate that reaches 400,000 deaths worldwide. Thus, the WHO sets a global target to eliminate HCV as a public health threat by 2030 [2]. Egypt has seroprevalence among the age group 15-59 years about 10% [3] which considered as the highest HCV prevalence worldwide, with genotype 4 dominating in more than 90% of all Egyptian patients.

In an attempt to control the situation in Egypt, the Ministry of Health established the National Committee for Control of Viral Hepatitis (NCCVH) which set a national treatment program aiming to decline the prevalence to less than 1% by 2030. In 2014, the National Committee provided Sofosbuvir and other direct-acting antiviral (DAA) agents in a very low cost for patients treated through the national program [4]. Sofosbuvir is a uridine nucleotide analog inhibitor of the viral NS5B polymerase required for viral replication. It interferes with the nascent viral RNA arresting its replication.

Mitochondrial dysfunction has been reported as an off-target effect of most DAA agents [5]. These agents exert mitochondrial toxicity through inhibiting mitochondrial DNA polymerase γ (pol γ) encoded by POLG gene, thus halting mitochondrial DNA (mtDNA) replication which leads to mtDNA depletion, increased reactive oxygen species (ROS) production and decreased synthesis of electron transport chain (ETC) proteins [6]. Mitochondrial homeostasis is maintained by the coordination between mitochondria biogenesis and mitochondrial degradation (mitophagy). Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) control the mitochondrial biogenesis through stimulating the expression of a series of nuclear transcription factors including nuclear respiratory factor-1 (NRF-1) which increases the expression of the mitochondrial transcription factor A (Tfam), the final effector of mtDNA transcription and replication [7]. Regarding mitochondrial biogenesis, the antiretroviral drugs have an impact on mtDNA copy number (mtDNA-CN) and the main regulators of mitochondrial biogenesis, PGC-1α, and NRF-1 [8]. These mitochondrial effects may interfere and impact the functions of the tissues depending on the type of the affected tissues and also depending on the developmental age.

The strategy of the National Committee for Control of Viral Hepatitis (NCCVH) to control HCV infection in Egypt was to treat every infected individual with DAA especially Sofosbuvir. This mass treatment strategy during the last years involved many of the young, infected females of childbearing age which attract our attention to the possible long lasting adverse effects of Sofosbuvir on the pregnancy and offspring of exposed mothers. Our previous work indicated disturbed mitochondrial biogenesis and functions in different tissues of young female rats exposed to the therapeutic dose of Sofosbuvir for three months. The ovarian tissue appears to be the most affected organ followed by the hepatic tissue and the skeletal muscles which means that Sofosbuvir-exposure not only affect the health of the exposed females but also affect the future offspring of these females (unpublished data).

During pregnancy, the in-utero environment is the vital element that programs to a large extent the health of an individual throughout life because the mammalian fetus is completely dependent on the nutrients supplied by its mother, and any change in this supply or shift in maternal metabolic status can alter fetal development structurally and functionally. This effect has been described as “fetal origin of adult disease” [9]. So, we designed this study to examine the possible effects of the pre-conception exposure of young female rats to Sofosbuvir on the pregnancy outcome and the mitochondrial biogenesis and function in different fetal tissues and placental tissue prenatally.

2. Materials And Methods

2.1. Experimental animals

The study was conducted on 20 female albino rats 2 months old were used. Rats were obtained from the animal house of the Medical Research Institute, Alexandria University, Egypt. Animals were kept 5 per cage at 23 °C in a 12 h light/12 h dark cycle under good hygienic conditions and standard humidity with access to food and water.

2.2. Ethical statement

The study fulfills the ARRIVE Guidelines for reporting animal research and a completed ARRIVE guidelines checklist is appended in S1 Checklist. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC)-Alexandria University, Egypt in 2020 (AU0122012113). All steps were performed following the guidelines for the care and use of laboratory animals (USA National Institute of Health Publication No 80-23, revised 1996) and all efforts were made to reduce the distress of rats during the whole experimental period.

2.3. Drug

Sofosbuvir was available in the form of tablets with the trade name 'Gratisovir' (a product of Pharco Pharmaceuticals, Alexandria, Egypt), Each tablet contains 400 mg of Sofosbuvir; these tablets were dissolved in distilled water and given to rats orally by gastric tube in a dose of 4 mg/kg/day for 3 months.

2.4. Experimental design

The animals were classified into two groups according to the treatment they were receiving:

- **Group I** (Control group): 10 healthy female rats that were maintained under normal diet and received placebo for 3 months.
- **Group I**I (exposure group): 10 healthy female rats that were supplemented with 4mg/kg of Sofosbuvir for 3 months [10].

Induction of pregnancy:

- Pregnancy was induced in control and Sofosbuvir-exposed females by mating with healthy male rats overnight. The next day was considered day 0 of pregnancy (gestational Day 0).
- At gestational day 17 (GD 17), 10 pregnant females of each group were sacrificed by cervical dislocation under deep anesthesia with ketamine 100 mg/kg and xylazine 10 mg/kg.

2.5 Collection of samples

Fetuses with their membranes and placentas were quickly dissected out of the uterine horns. The number of resorbed (dead) fetuses for each pregnancy was noted and excluded from the study. Each fetus and its membrane were separated by gentle dissection and rinsed carefully in phosphate buffer saline (PBS). Overall growth and differentiation of the embryos were quantified by direct measurement of crown-rump length. The weights of fetuses and placentas were recorded. The liver, muscle, and placental tissues were obtained, washed, and each organ was divided into 3 aliquots; the first one used for DNA isolation for the assessment of mitochondria DNA copy number (mtDNA-CN), the second used for total RNA isolation for the assessment of gene expression, and the third aliquot was homogenized in phosphate buffered saline (0.1 M, pH 7.4) in ratio1:9 and centrifuged at 10000×g for 10 min at 4 C and the supernatant was stored

in aliquots for subsequent determinations of total protein level by Lowery method, malondialdehyde (MDA), Glutathione and the protein levels of NADH dehydrogenase subunit-5 (ND5).

2.6 Tissues contents of NADH dehydrogenase subunit-5 using ELISA

The supernatant was used for NADH dehydrogenase subunit-5 (ND5) determination using ELISA assay (Chongqing Biospes Co.,China) according to the manufacturer's instructions. The total protein concentration was determined using Lowry's method [11].

2.7 Tissues contents of total reduced and oxidized glutathione:

Glutathione (GSH) and glutathione disulphide (GSSG) were assayed using the method of Griffith which depends on the oxidation of GSH by 5,5`-dithiobis-(2-nitrobenzoic acid) (DTNB) to yield GSSG and 5-thio-2-nitrobenzoic acid (TNB). Oxidised GSSG is reduced enzymatically by the action of glutathione reductase and NADPH to regenerate GSH. The rate of TNB formation is monitored at 412 nm and is proportional to the sum of GSH and GSSG present in the sample [12].

2.8 Determination of malondialdehyde (MDA) as thiobarbituric acid reactive substances (TBARS):

Malondialdehyde was determined according to the method of Draper and Hadley. The tissues samples are heated with thiobarbituric acid (TBA) at low pH. The resulting pink chromogen has a maximal absorbance at 532 nm [13].

2.9 Tissue expression of PGC-1α, Tfam, NRF-1, NF-KB and POLG

Thirty mg of liver, muscle, or placental tissues were used for total RNA extraction using the miRNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions and the concentration and integrity of extracted RNA were checked using nanodrop. The reverse transcription of the extracted RNA was performed using Reverse transcription (RT) was performed by TOPscript™ RT DryMIX kit (dT18/dN6 plus) (Enzynomics, Korea) according to the manufacturer instructions. The tissues expression of PGC-1α, Tfam, NRF-1, nuclear factor kabba B (NF-KB), and DNA polymerase gamma (POLG) were quantified in the cDNA by CFX Maestro™ Software (Bio-Rad, USA) using QuantiNova™ SYBR® Green PCR Kit (Qiagen, Germany). Quantitative PCR amplification conditions were adjusted as an initial denaturation at 95°C for 10 minutes and then 45 cycles of PCR for amplification as follows: Denaturation at 95 °C for 20 s, annealing at 55 °C for 20 s and extension at 70 °C for 15 s. The housekeeping gene 18S rRNA was used as a reference gene for normalization. The primers used for the determination of rat genes are presented in Table 1. The relative change in mRNA expression in samples was estimated using $2^{-\Delta\Delta Ct}$ method.

2.10 Tissues mitochondrial DNA copy number (mtDNA-CN)

A qRT-PCR assay was used to estimate relative levels of mtDNA-CN in the total DNA extracted from the samples. mtDNA-CN was determined by calculating the ratio of PCR amplicons of mitochondrial

sequence (mtDNA) to that of a single nuclear gene (n-PGC1 alpha). In this study, total DNA was isolated from the different tissues using DNeasy kit (Qiagen, USA) according to the manufacturer instructions. A specific primer pair for mtDNA and a primer pair for nuclear PGC-1α gene (Table 1) were used. PCR reactions were carried out using SYBR Green PCR Master Mix (Qiagen, Germany), 0.5 μM of each primer pair, and 50 ng genomic DNA under the following conditions: 95˚C for 10 min followed by 40 cycles of 95˚C for 15 sec, 60˚C for 30 sec and 72˚C for 30 sec. The relative mtDNA-CN was calculated using the equation $2^{-\Delta Ct}$ ($\Delta Ct = Ct_{mtDNA} - Ct_{n\text{-}PGC\text{-}1\alpha}$) according to a previous report [14].

2.11. Statistical analysis

Data were analyzed using SPSS software package version 18.0 (SPSS, Chicago, IL, USA). The data were expressed as mean ± SE and analyzed using Student independent-T test to compare between different groups. The p value was assumed to be significant at $p < 0.05$ [15].

3. Results

3.1. Pregnancy outcome

The pregnancy outcome of control and Sofosbuvir-exposed mothers is illustrated in Table 2. Sofosbuvir-exposed mothers showed decreased number of viable fetuses per letter by about 29% compared with control pregnancies. Moreover, the pregnancies of Sofosbuvir-exposed mothers have marked increase in the resorbed (dead) fetuses by about 375% compared with the pregnancies of control mothers.

The placentas of fetuses of Sofosbuvir-exposed mothers were significantly heavier than the placentas of the fetuses of control mothers by about 35%. The fetal growth was assayed by fetal weight and crown-rump length (CRL). The results indicated that the fetuses of Sofosbuvir-exposed mothers are significant heavier (increased weight) and longer (long CRL) than by about (CRL) by about 118% and 37%, respectively, compared with the fetuses of control mothers.

3.2 Malondialdehyde (MDA) content in different tissues

The content of MDA was significantly increased in the all tissues of fetuses of Sofosbuvir-exposed mothers by about 74% in the liver, 144% in muscle and 667% in placenta compared with fetuses of control mothers (Table 3).

3.3 Glutathione contents in different tissues

Fetuses of Sofosbuvir-exposed mothers showed significant decrease in total glutathione (t GSH) and reduced glutathione (GSH) in all tissues compared with fetuses of control mothers **(Figure 1a, 1b)** especially in fetal liver.

Fetuses of Sofosbuvir-exposed mothers showed significant increase in oxidized glutathione (GSSG) in placental tissue however showed no significant changes were observed in liver and muscle tissues compared with fetuses of control mothers **(Figure 1c).**

Fetuses of Sofosbuvir-exposed mothers showed significantly decrease in GSH/GSSG in all tissues compared with fetuses of control mothers especially in placental tissues **(Figure 1d).**

3.4 NADH dehydrogenase subunit-5 (ND5) content in different tissues

The content of ND5 was significantly lower by about 50% in the liver of fetuses of Sofosbuvir-exposed mothers compared with fetuses of control mothers, while fetal muscle have significant higher ND5 content than the fetuses of control mothers by about 53%. The placental tissues of fetuses of Sofosbuvir-exposed mothers show non-significant decrease in ND5 content by about 17% compared with the control placentas **(Figure 2).**

3.5 Mitochondrial DNA copy number (mtDNA-CN) in different tissues

The fetal placentas of fetuses of Sofosbuvir-exposed mothers have significantly higher mtDNA-CN by about 43% compared with fetuses of control mothers. The fetal livers show mild but non-significant increase by about 24% compared with fetuses of control mothers. In contrast, the fetal muscle of fetuses of Sofosbuvir-exposed mothers have significantly lower mtDNA-CN compared with the fetuses of control mothers by about 29% **(Figure 3)**.

3.6 Expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α)

The expression of PGC-1α was significantly induced in the placental tissues of fetuses of Sofosbuvir-exposed mothers to be 2.5-fold compared with fetuses of control mothers. In contrast, the expression of PGC-1α was significantly suppressed in the liver tissues of fetuses of Sofosbuvir-exposed mothers by about 68% compared with fetuses of control mothers. The expression of PGC-1α showed mild but non-significant increase by about 28% in the muscle of fetuses of Sofosbuvir-exposed mothers compared with fetuses of control mothers **(Figure 4)**.

3.7 Expression of nuclear respiratory factor-1 (NRF-1)

The expression of NRF-1 was significantly suppressed in liver tissues of fetuses of Sofosbuvir-exposed mothers compared with fetuses of control mothers by about 56%. In contrast, NRF-1 expression was significantly upregulated in the skeletal muscle tissues of fetuses of Sofosbuvir-exposed mothers to be about 4-fold the control value. The placental tissues show no significant change in the expression of NRF-1 in the fetuses of Sofosbuvir-exposed mothers compared with the fetuses of control mothers **(Figure 5)**.

3.8 Expression of mitochondrial transcription factors-A (Tfam)

The expression of Tfam was significantly suppressed in the liver and skeletal muscle of fetuses of Sofosbuvir-exposed mothers by about 85% compared with fetuses of control mothers, while the placental tissues of fetuses of Sofosbuvir-exposed mothers have lower but not significant Tfam expression compared with the control placentas (**Figure 6)**.

3.9 Tissue expression of mitochondrial DNA polymerase gamma (POLG)

The expression of POLG was significantly suppressed in placental tissues of fetuses of Sofosbuvir-exposed mothers by about 45% compared with fetuses of control mothers. In contrast, the expression of POLG was significantly upregulated in skeletal muscle tissues of fetuses of Sofosbuvir-exposed mothers by about 94% compared with the fetuses of control mothers. The liver tissues of fetuses of Sofosbuvir-exposed mothers show non-significant decrease in the expression of POLG compared with the fetuses of control mothers (**Figure 7)**.

3.10 Tissue expression of nuclear factor kabba B expression (NF-κB)

The expression of NF-κB was significantly induced in the all tissues of fetuses of Sofosbuvir-exposed mothers to be 2.5-fold in liver, 3 fold in muscle, 2 fold in placenta compared with fetuses of control mothers (**Figure 8)**.

3.11 Correlation studies

The statistical analysis using Pearson correlation reveals that PGC-1α expression was positively correlated with NRF-1 expression (r= 0.911, p□0.001, Figure 9) and Tfam expression (r= 0.681, p=0.030, Figure 9) in placental tissue of fetuses of Sofosbuvir-exposed mothers.

4. Discussion

The possible mitochondrial toxicity of sofosbuvir is underestimated and need intense investigations given in account the mass treatment by sofosbuvir at the national level like in Egypt. The present study aimed to assess the possible mitochondrial side effects of the preconception exposure of young female rats on the prenatal fetal tissues and placentas. The results raise serious concerns about the safety of sofosbuvir treatment in young female because it affects the pregnancy outcome and the mitochondrial biogenesis and function in fetal and placental tissue prenatally.

The preconception exposure of young female rats to Sofosbuvir results in significant decline in the pregnancy outcomes as indicated by lower numbers of viable embryos per letter and higher rats of fetal resorption (dead fetus). Also, fetal size and the fetal and placental weights are significantly higher than the control values. These effects may be related to the decreased quality of the oocytes due to the three months exposure to Sofosbuvir that may impairs the mitochondrial biogenesis and functions in the ovarian tissues of the mothers before the pregnancy which ultimately results in decreased fertility. This assumption is confirmed by our previous work indicated disturbed mitochondrial biogenesis and functions in the ovarian tissue due to treatment with Sofosbuvir (unpublished data). The low ovarian

mtDNA-CN is a marker of poor oocyte quality, which may lead to poor embryonic development [16]. The maternal mitochondrial dysfunctions and impaired biogenesis may be transmitted to following generations and may cause many diseases [17]. Also, the possible direct effects of the Sofosbuvir administered before pregnancy cannot be ruled out since animal studies prove that Sofosbuvir metabolites cross the placenta and are excreted in the milk of nursing animals [18].

The placental defects may be another contributing factor for the decline in pregnancy outcomes and fetal abnormalities. The placenta is the first organ formed in pregnancy and it connects the maternal and embryonic circulations to facilitate the fetal supply with maternal nutrients [19]. The placentomegaly observed in the pregnancies of Sofosbuvir-exposed mothers may indicate a state of chronic inflammation which confirmed in our study by induced placental expression of NF-κB. The placental inflammation may compromise its function as a selective barrier for the transport of nutrients and others into the fetus. In our study, placental inflammation was associated with significant increase in lipid peroxidation marker (MDA), and oxidized glutathione (GSSG) and significant drop in total and reduced GSH. These changes results in shifting the redox status from reducing environment (high GSH/GSSG ratio) into more oxidizing environment (low GSH/GSSG ratio). High concentrations of GSH are associated with a reducing environment and increased proliferation, while cell death is initiated by an oxidizing environment [20]. Thus, the redox status of the placental tissues of Sofosbuvir-exposed mothers indicate a state of cell death and inflammation. The structural changes and maladaptation in the placenta may cause inflammatory shock to the fetus that may results in long-term adverse outcomes, including asthma, cerebral palsy, abnormal neurodevelopment, and autism spectrum disorder [21,22].

The observed changes in fetal and placental size and weight due to the maternal exposure to Sofosbuvir are associated with significant changes in the mitochondrial biogenesis and functions in fetal tissues and placentas and significant changes in the patterns of gene expression of genes controlling mitochondrial biogenesis and functions. However, the patterns of changes are not similar between the different organs and placenta.

Regarding the mitochondrial DNA copy number, an indicator of mitochondrial biogenesis, the placental tissues of Sofosbuvir-exposed mothers were increased significantly, the fetal liver showed no significant change, however the fetal muscle was declined significantly. The differential effects of the pre-conception maternal exposure to Sofosbuvir on the fetal tissues may explained by the fact that the different tissues are not homogeneous regarding the mtDNA-CN depending on the energy demand and the metabolic requirements. This explanation agrees with Pejznochova and colleagues who assumed that the non-homogenous distribution of mtDNA between fetal tissues is attributed to the differences in their metabolic roles, and that mtDNA-CN in fetal tissues is considered tissue-specific and depends on energy requirements as well as the stage of development [23]. This explains the variation in mtDNA-CN in different sampled tissues in our study and the differential effects of

In the present study, the increased placental mtDNA copy number is associated with significant upregulation in the expression of PGC-1α and significant downregulation in the expression of POLG. The

placental inflammation and oxidative stress may lead to mitochondrial proliferation to compensate the disrupted cellular bioenergetics. On the other hand, increased placental mitochondrial ROS may cause direct damage to mtDNA, thus, inhibiting mitochondrial biogenesis. However, in an attempt to maintain normal fetal growth, either increased or decreased mitochondrial biogenesis could take place [24].

Besides its key role as the main regulator of mitochondrial biogenesis, PGC-1α has been recognized as a powerful regulator of angiogenesis which is fundamental to the development of a healthy placenta in normal pregnancy. It may be possible that PGC-1α has a key role in the imbalance of angiogenic factors observed in pre-eclampsia. Delany and colleagues suggested that PGC-1α may be implicated in the pathogenesis of pre-eclampsia [25]. Vishnyakova et al. found increased placental mitochondrial content in early-onset but not late-onset pre-eclampsia, suggesting that the different pathophysiology leads to differences in mitochondrial response [26]. The maintenance of mitochondrial biogenesis during the critical period of fetal development is very crucial because the increased mitochondrial biogenesis during pregnancy is required to maintain metabolic activity of the placenta. Disruption of this process could increase the production of ROS and oxidative stress which subsequently leads to placental insufficiency and damage that is may be manifested as pre-eclampsia or intrauterine growth restriction [27].

In fetal muscle, the decline in mtDNA copy number is associated with marked induction of ND5 content and the expression of NRF-1 and POLG expression. These inductions may be an adaptive response to overcome the decline in mitochondrial biogenesis. Under normal physiological conditions, NRF-1 is widely expressed, and the highest expression has been recognized in skeletal muscles [28]. Alone, NRF-1 was not enough to induce mitochondrial biogenesis, which was consistent with our findings. In this context, Baar et al., reported that the increased NRF-1 was not sufficient to initiate the expression of the proteins required for the assembly of functional mitochondria [29]. Also, the mutations in POLG can cause either early childhood or later-onset syndromes resulting from mtDNA deletions [30].

Mitochondrial biogenesis is accompanied with skeletal myogenesis. Therefore, prenatal mitochondrial dysfunction and decreased mitochondrial biogenesis in skeletal muscles may manifest later in life, causing long-term consequences on energy homeostasis [31]. Kang et al. found direct relation between Tfam and mtDNA, as heterozygous mutations of Tfam negatively affects mtDNA-CN, whereas homozygous mutation is embryonically lethal. Tfam heterozygous cells produce more inflammatory cytokines due to mitochondrial stress signaling. Hence, downregulation of Tfam expression resulted in mtDNA stress signaling. Levels of Tfam directly control mtDNA content, thus, any post-translational modifications that disrupt either the turnover or stability of Tfam could substantially impact the regulation of mtDNA abundance [23, 32].

In fetal liver, the pre-conception exposure of young females to sofosbovir contributed to mitochondrial dysfunction as indicated by significant decline in the content of ND5. NADH dehydrogenase, complex I, is the first and largest of the ETC complexes that has a pivotal role in energy metabolism as it is the main entry point for electrons to the ETC, hence, considered the rate-limiting step in overall respiration.

Mutations in the genes encoding the complex I subunits have been associated with ETC disturbances and ROS production [33]. Although the main pathway regulating mitochondrial biogenesis has been downregulated, mtDNA-CN, which represents a marker of mitochondrial biogenesis, does not significantly changed but showed mild decline.

In liver, mitochondria regulate lipid and amino acid metabolism, energy production and the urea cycle, as well as regulating the innate immune response to control [34]. Sofosbuvir exposure causes fetal liver inflammation as shown by an increase in NF-KB expression, also increased oxidative stress as demonstrated by an elevation in MDA content and a decline in the GSH/GSSG ratio, which can contribute to the development and progression of liver diseases.

The observed prenatal effects of pre-conception maternal exposure to Sofosbuvir may have long-term adverse effects through impacting the health of the offspring in the adult life. The mechanism(s) of pre-conception Sofosbuvir exposure-induced changes in the pregnancy outcomes and fetal and placental tissues is (are) unclear and needs intensive study. However, we can suggest a different possible mechanism that may participate: (1) Sofosbuvir may directly or indirectly affect the quality of the ova through suppressing the gene expression of PGC-1α and/or Tfam that drives fetal abnormalities developed later after pregnancy, (2) Sofosbuvir may induce placental abnormalities and oxidative stress early in pregnancy that may affect the nutrient supply to the fetus which results in increased risk of mtDNA mutations, leading to mitochondrial dysfunction, and (3) Sofosbuvir may affect the hormonal homeostasis pre-conception and during pregnancy that may affect the fetal metabolism and development.

5. Conclusion

From the present study we can concluded that the preconception exposure of young female rats to sofosbuvir affect the pregnancy outcomes, decrease fertility, and impact the mitochondrial biogenesis and functions in the prenatal fetal liver, skeletal muscle and placental tissues through changing the pattern of fetal and placental expression of the genes controlling mitochondrial biogenesis and functions.

Declarations

Author's contribution:

Maher A. Kamel: Proposed research plan, analyzed and interpreted the results, and edited and reviewed manuscript.

Rana HM. Khafaga: Conceived and designed the experiments, and critical revision of the manuscript.

Maryam.M.Abdel-Aziz: Performed practical work and wrote manuscript.

Sara.A.Shaker: Supervised the practical part, data analysis and interpretation, and writing of the manuscript.

Hala A. Hafez: Acquisition of data, data analysis and interpretation, and critical revision of the manuscript.

Shimaa A. Mahmoud: Supervise the practical part and contributed to the analysis and manuscript critique.

References

1. Spearman, C. W., Dusheiko, G. M., Hellard, M., & Sonderup, M. Hepatitis C. *The Lancet*, *394*(10207), 1451–1466 (2019).
2. Dore, G. J., & Bajis, S. Hepatitis C virus elimination: laying the foundation for achieving 2030 targets. *Nature Reviews Gastroenterology and Hepatology*, *18*(2), 91–92 (2021).
3. Omran, D., et,al. Towards hepatitis C virus elimination: Egyptian experience, achievements and limitations. *World Journal of Gastroenterology*, *24*(38), 4330–4340 (2018).
4. Gomaa, A., Allam, N., Elsharkawy, A., El Kassas, M., & Waked, I. Hepatitis C infection in Egypt: prevalence, impact and management strategies. *Hepatic Medicine: Evidence and Research*, *9*, 17–25 (2017).
5. Kim, S. J., et,al. Ginsenoside Rg3 restores hepatitis C virus–induced aberrant mitochondrial dynamics and inhibits virus propagation. *Hepatology*, *66*(3), 758–771 (2017).
6. Stoker, M. L., Newport, E., Hulit, J. C., West, A. P., & Morten, K. J. Impact of pharmacological agents on mitochondrial function: a growing opportunity? *Biochemical Society Transactions*, *47*(6), 1757–1772 (2019).
7. Popov, L.-D. Mitochondrial biogenesis: An update. *Journal of Cellular and Molecular Medicine*, *24*(9), 4892–4899 (2020).
8. Viengchareun, S., et,al. Mitochondrial toxicity of indinavir, stavudine and zidovudine involves multiple cellular targets in white and brown adipocytes. *Antiviral Therapy*, *12*(6), 919–929 (2007).
9. Kamel, M. A. Prenatal Effects of Different Intra-Uterine Milieus on Fetal Glucose Sensing Mechanisms during Gestation in Rats. *Journal of Diabetes & Metabolism*, *3*(3), 181. doi: 10.4172/2155-6156.1000181 (2012).
10. Issa, N.M.,& El-sherief, N.M. Histological and Immunohistochemical Studies on the Cornea and Retina of Sofosbuvir Treated Rats. *Austin J Anat,* 4 (2),1068 (2017).
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry,* 193(1), 265–275 (1951).

12. Griffith, O.W. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Analytical biochemistry 106(1), 207–212 (1980).
13. Draper, H. H., Hadley, M., Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*, 186, 421-425 (1990).
14. Ekstrand, M., et,al. Mitochondrial transcription factor A regulates mtDNA copy number in mammals. *Hum Mol Gen,*13(9), 935–944 (2004).
15. Hagen, S. SPSS in Practice . *Nurse Researcher*, *10*(2), 86–87 (2002).
16. May-Panloup, P., et,al. Ovarian ageing: the role of mitochondria in oocytes and follicles. *Human Reproduction Update*, *22*(6), 725–743 (2016).
17. Chiaratti, M. R., et,al. Oocyte mitochondria: role on fertility and disease transmission. *Animal Reproduction*, *15*(3), 231–238 (2018).
18. Spera, A. M., Eldin, T. K., Tosone, G., & Orlando, R. Antiviral therapy for hepatitis C: Has anything changed for pregnant/lactating women? *World Journal of Hepatology*, *8*(12), 557–565 (2016).
19. Herrera, E., & Desoye, G. Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. *Hormone Molecular Biology and Clinical Investigation*, *26*(2), 109–127 (2016).
20. El-Bassiouni, E.A., Helmy, M.H., Abou Rawash, N., El-Zoghby, S.M., Kamel, M.A.,& Abou Rayah, A.N. Embryopathy in experimental diabetic gestation: assessment of oxidative stress and antioxidant defence. *British Journal of Biomedical Science,* 62(2),71-76 (2005).
21. Saad, M.I., et,al. Maternal obesity and malnourishment exacerbate perinatal oxidative stress resulting in diabetogenic programming in F1 offspring. J Endocrinol Invest, 39(6),643-655 (2016).
22. Goldstein, J. A., Gallagher, K., Beck, C., Kumar, R., & Gernand, A. D. Maternal-Fetal Inflammation in the Placenta and the Developmental Origins of Health and /Disease. *Frontiers in Immunology*, *11*, 1–14 (2020).
23. Pejznochova, M., et,al. Mitochondrial DNA content and expression of genes involved in mtDNA transcription, regulation and maintenance during human fetal development. *Mitochondrion*, *10*(4), 321–329 (2010).
24. Holland, O., et,al. Review: Placental mitochondrial function and structure in gestational disorders. *Placenta*, *54*, 2–9 (2017).
25. Delany, A., McCarthy, F., Walsh, S., & Kenny, L. PP053. The role of peroxisome proliferator-activated receptor gamma co-activator 1-alpha in pregnancy. *Pregnancy Hypertension*, *3*(2), 86 (2013).
26. Vishnyakova, P. A., et,al. Mitochondrial role in adaptive response to stress conditions in preeclampsia. *Scientific Reports*, *6*, 1–9 (2016).
27. Mandò, C., et,al. Placental mitochondrial content and function in intrauterine growth restriction and preeclampsia. *American Journal of Physiology - Endocrinology and Metabolism*, *306*(4), 404–413 (2014).
28. Roy, D., & Tamuli, R. NRF1 (nuclear respiratory factor 1). *Atlas of Genetics and Cytogenetics in Oncology and Haematology*, *13*(11), 861–864 (2009).

29. Baar, K., et,al. Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. *The FASEB Journal*, *17*(12), 1666–1673 (2003).
30. Cheng, K., et,al. Effects of Early Resveratrol Intervention on Skeletal Muscle Mitochondrial Function and Redox Status in Neonatal Piglets with or without Intrauterine Growth Retardation. *Oxidative Medicine and Cellular Longevity*, *2020*, 1–12 (2020).
31. Zou, T. D., et,al. Mitochondrial biogenesis is decreased in skeletal muscle of pig fetuses exposed to maternal high-energy diets. *Animal*, *11*(1), 54–60 (2017).
32. Kang, I., Chu, C. T., & Kaufman, B. A. The mitochondrial transcription factor TFAM in neurodegeneration: Emerging evidence and mechanisms. *FEBS Letters*, *592*(5), 793–811 (2018).
33. Sharma, L., Lu, J., & Bai, Y. Mitochondrial Respiratory Complex I: Structure, Function and Implication in Human Diseases. *Current Medicinal Chemistry*, *16*(10), 1266–1277 (2009).
34. Mansouri, A., Gattolliat, C.-H., & Asselah, T. Mitochondrial Dysfunction and Signaling in Chronic Liver Diseases. *Gastroenterology*, *155*(3), 629–647 (2018).

Tables

Table 1: Primers used for the PCR amplification

Gene	Accession number	primer sequence	
18S rRNA (Reference gene)	NR_046237.2	F:	GTAACCCGTTGAACCCCATT
		R:	CAAGCTTATGACCCGCACTT
PGC-1α	NM_031347.1	F:	GTGCAGCCAAGACTCTGTATGG
		R:	GTCCAGGTCATTCACATCAAGTTC
NRF-1	NM_001100708.1	F:	TTACTCTGCTGTGGCTGATGG
		R:	CCTCTGATGCTTGCGTCGTCT
Tfam	NM_031326.2	F:	CCCACAGAGAACAGAAACAG
		R:	CCCTGGAAGCTTTCAGATACG
POLG	NM_053528.1	F:	GGACCTCCCTTAGAGAGGGA
		R:	AGCATGCCAGCCAGAGTCACT
mtDNA	NC_040919.1	F:	AATGGTTCGTTTGTTCAACGATT
		R:	AGAAACCGACCTGGATTGCTC
n-PGC-1α	NM_031347.1	F:	ATGAATGCAGCGGTCTTAGC
		R:	5'- AACAATGGCAGGGTTTGTTC
NF-κB (P65)	NM_199267.2	F:	CAGGACCAGGAACAGTTCGAA
		R:	CCAGGTTCTGGAAGCTATGGAT

Table 2: Pregnancy outcome of control and Sofosbuvir-exposed mothers

Groups	Number of resorbed fetus/letter	Number of viable fetus/letter	Weight (g)		Fetal Crown-rump length (cm)
			Fetus	Fetal placenta	
Control	0.8 ± 0.2	13.2 ± 1.56	0.77 ± 0.01	0.38 ± 0.008	1.7 ± 0.01
Sofosbuvir exposed	3.8* ± 0.96	9.4 ± 1.43	1.68* ± 0.05	0.47* ± 0.01	2.33* ± 0.03
% of change from control	375	-28.8	118.1	35.13	37

Data were illustrated as Mean ± SE

Number of fetuses in each group = 10

*$p < 0.05$, indicating a statistically significant difference when compared with control group using independent sample t-test.

g: gram

cm: centimeter

Table 3: The change of Malondialdehyde content (nmol/mg protein) in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers.

Groups	MDA (nmol/mg protein)		
	Fetal liver	Fetal muscle	Fetal placenta
Control	0.78 ± 0.12	1.7 ± 0.1	0.30 ± 0.01
Sofosbuvir exposed	1.36* ± 0.04	4.15* ± 0.06	2.33* ± 0.12
% of change from control	74.3	144.1	667.6

Data were illustrated as Mean ± SE

Number of fetuses in each group = 10

*$p < 0.05$, indicating a statistically significant difference when compared with the control group using independent sample t-test.

MDA: Malondialdehyde

Figures

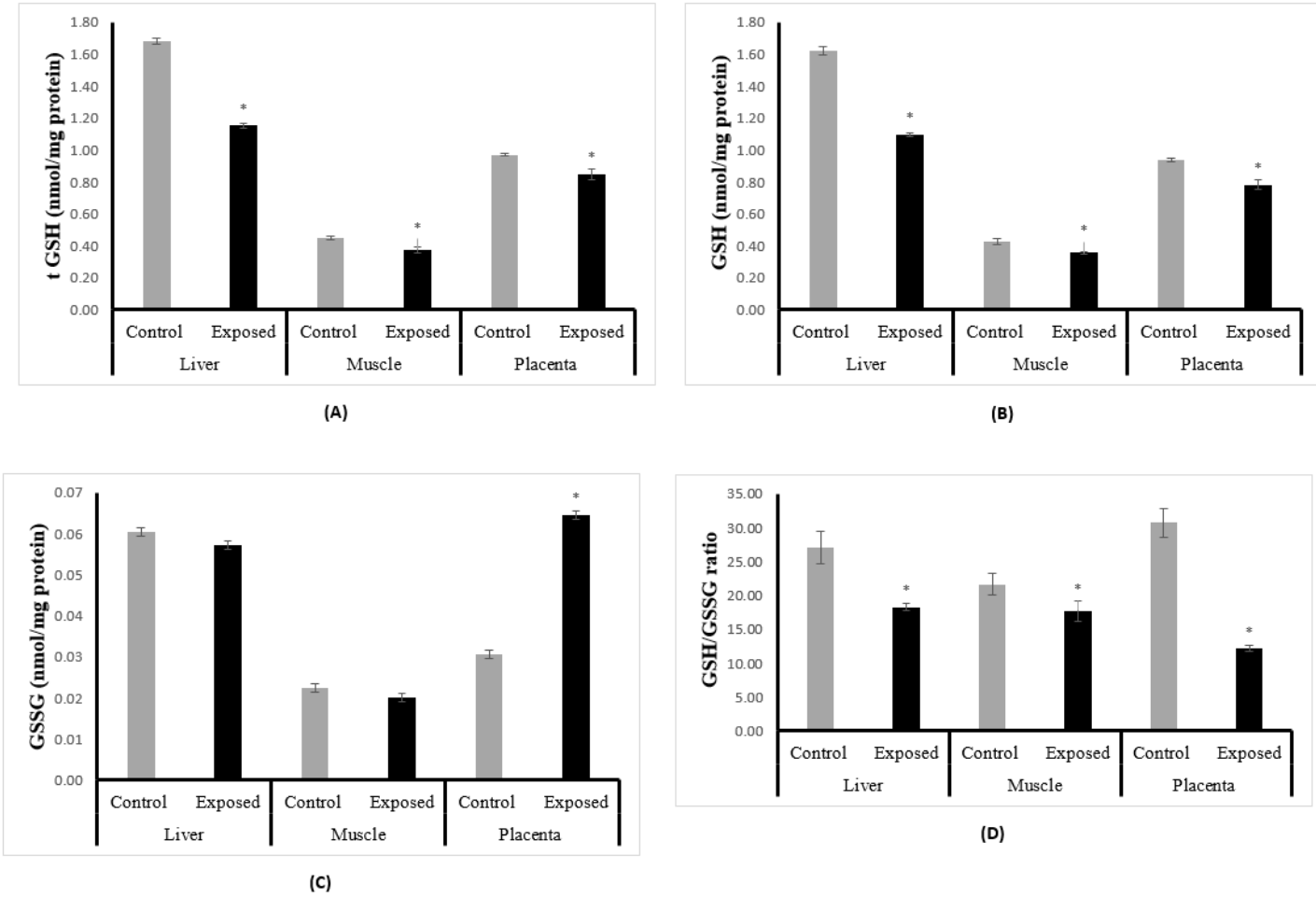

Figure 1

The change of glutathione content in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. Data were illustrated as Mean ± SE Number of fetuses in each group = 10 *p < 0.05, indicating a statistically significant difference when compared with control group using independent sample t-test. t GSH: Total glutathione GSH: reduced glutathione GSSG: Oxidized glutathione

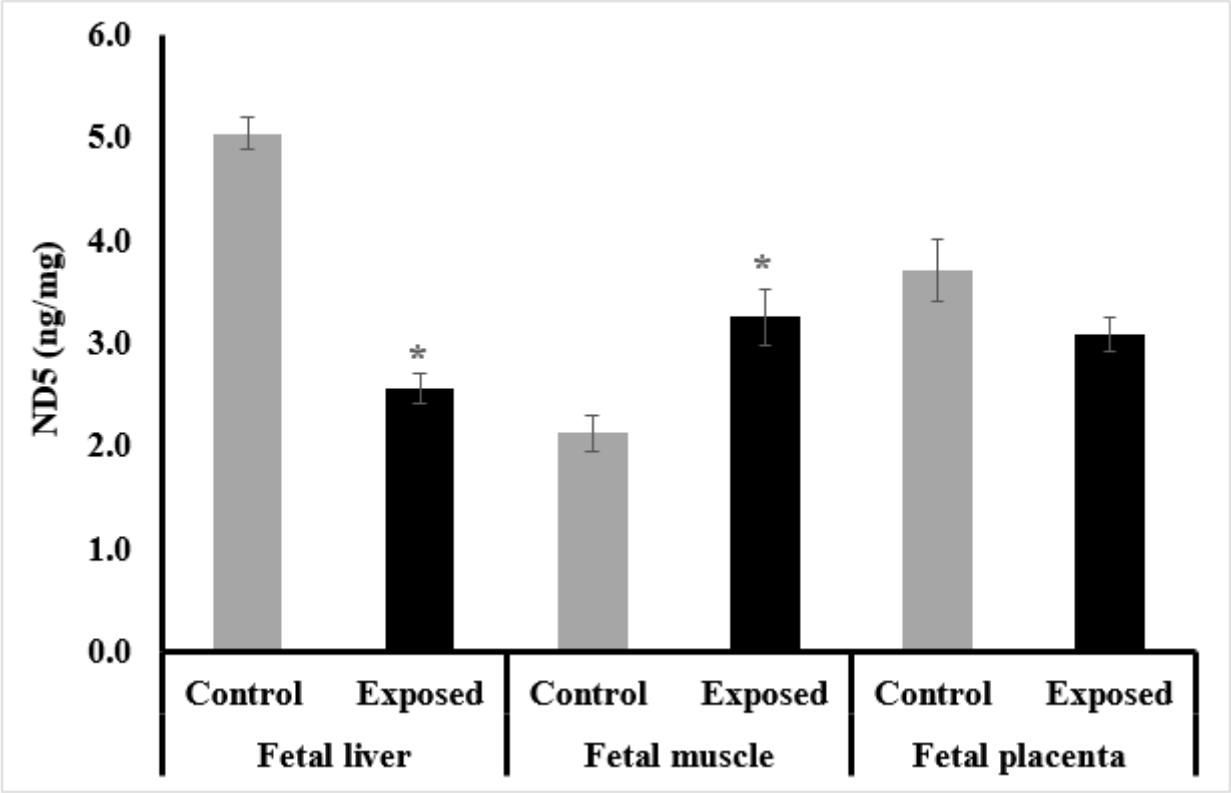

Figure 2

The change of ND5 content in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. Data were illustrated as Mean ± SE Number of fetuses in each group = 10 *p < 0.05, indicating a statistically significant difference when compared with control group using independent sample t-test. ng/mg: nanogram/milligram ND5: NADH dehydrogenase subunit-5

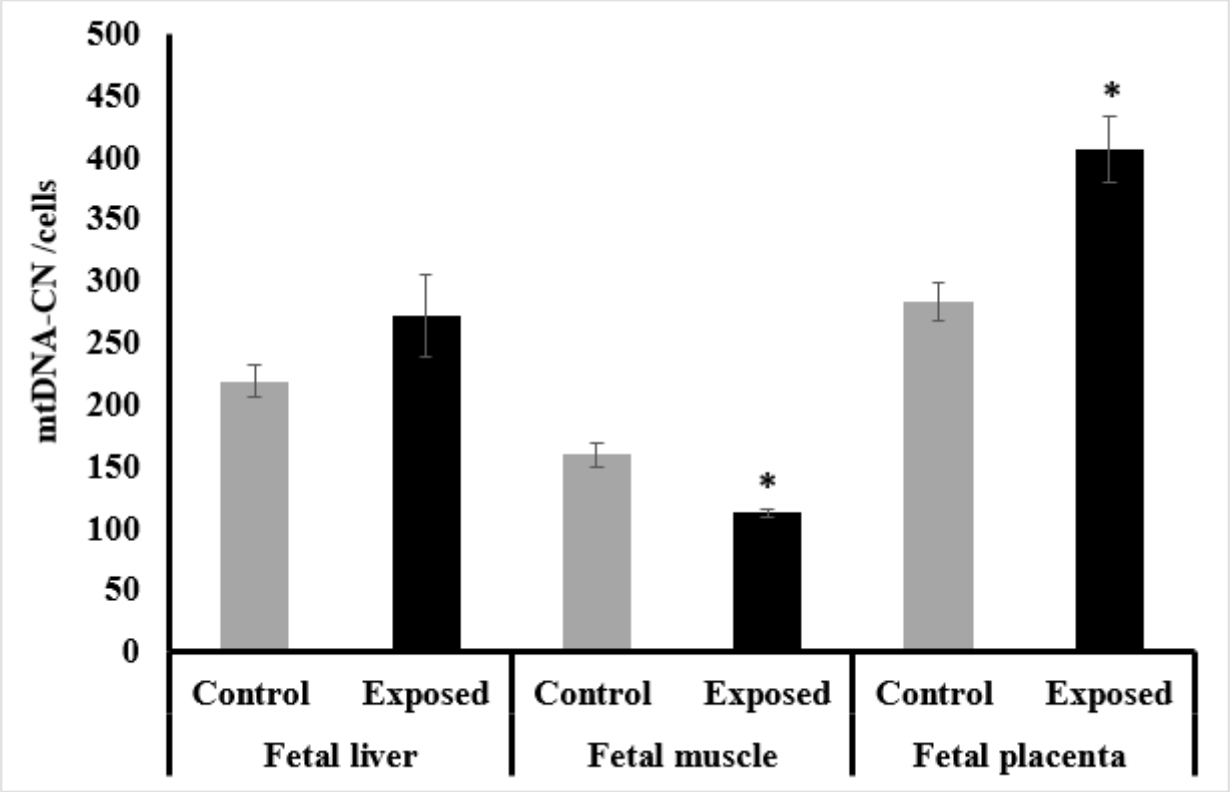

Figure 3

The change of mtDNA-CN in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. Data were illustrated as Mean ± SE Number of fetuses in each group = 10 *p < 0.05, indicating a statistically significant difference when compared with control group using independent sample t-test. mtDNA-CN: mitochondrial DNA copy number

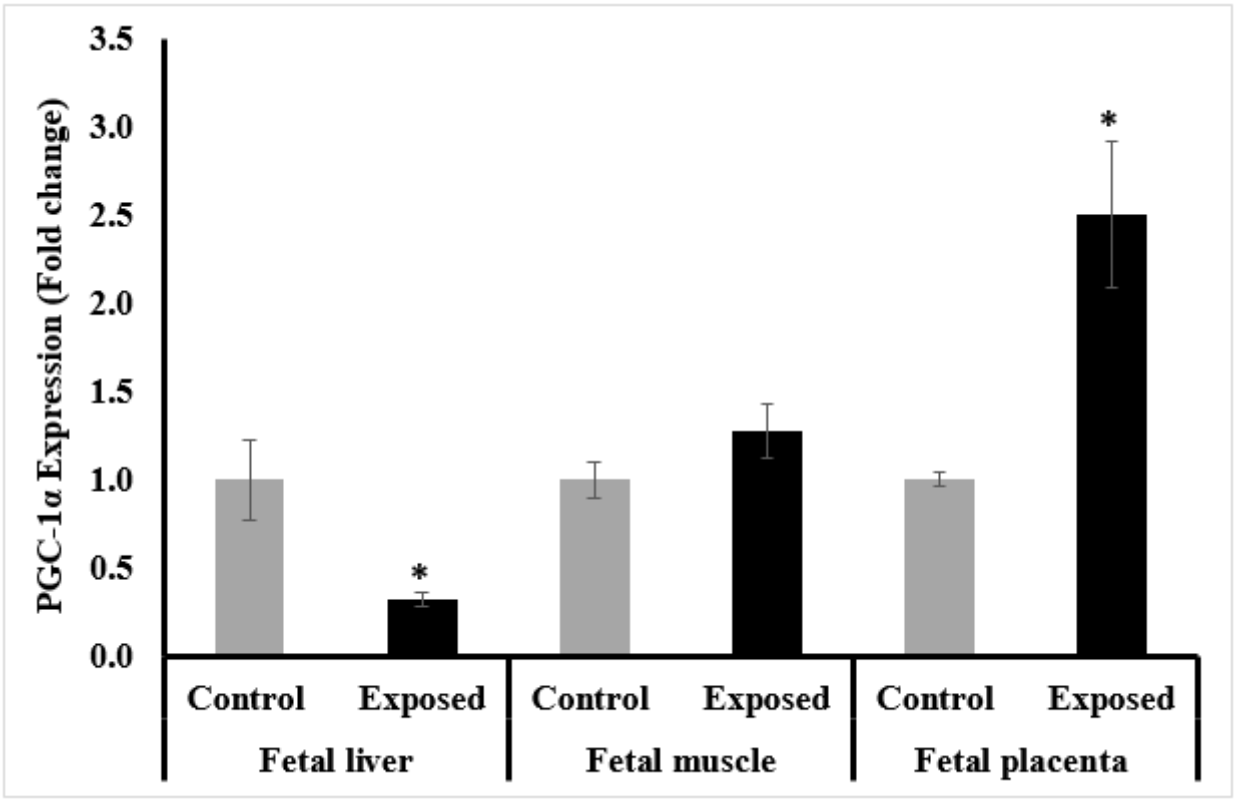

Figure 4

The change of PGC-1α gene expression (Fold change) in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. Data were illustrated as Mean ± SE Number of fetuses in each group = 10 *p < 0.05, indicating a statistically significant difference when compared with control group using independent sample t-test. PGC-1α: peroxisome proliferator-activated receptor gamma coactivator-1 alpha

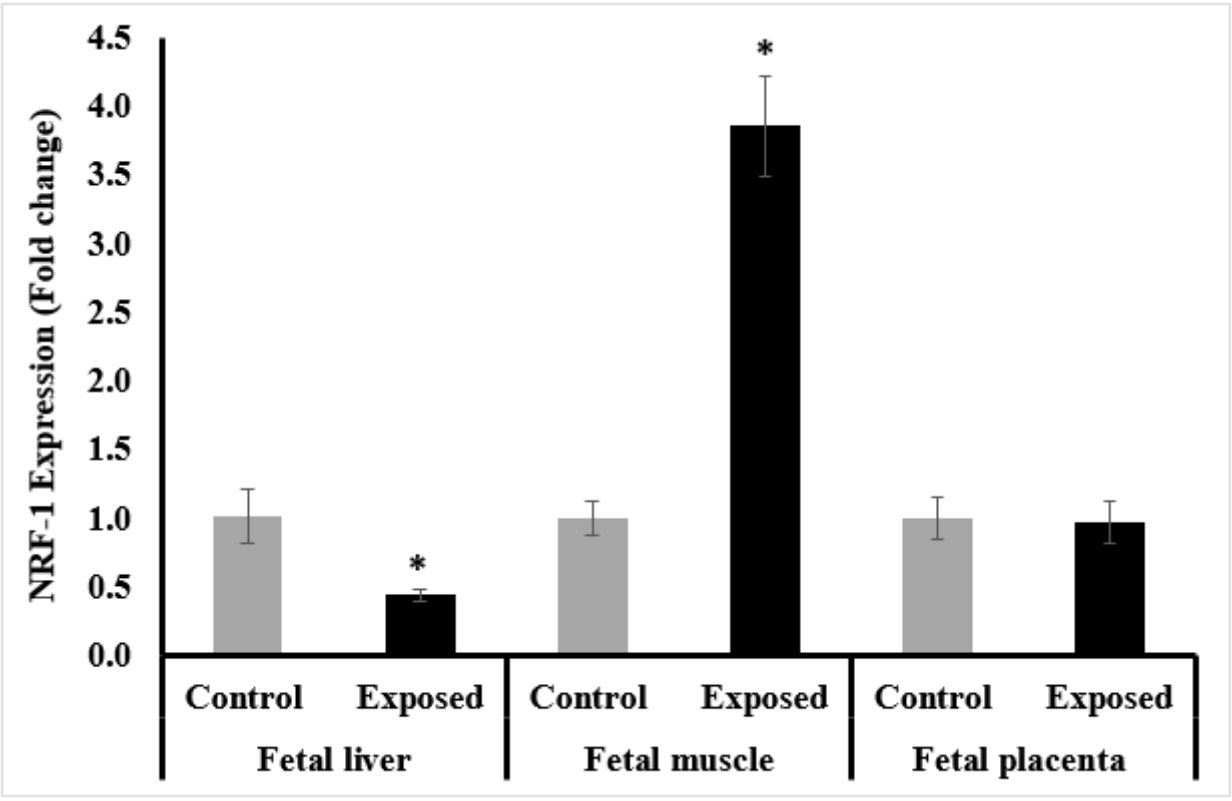

Figure 5

The change of NRF-1 gene expression (Fold change) in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. Data were illustrated as Mean ± SE Number of fetuses in each group = 10 *p < 0.05, indicating a statistically significant difference when compared with control group using independent sample t-test. NRF-1: nuclear respiratory factor-1

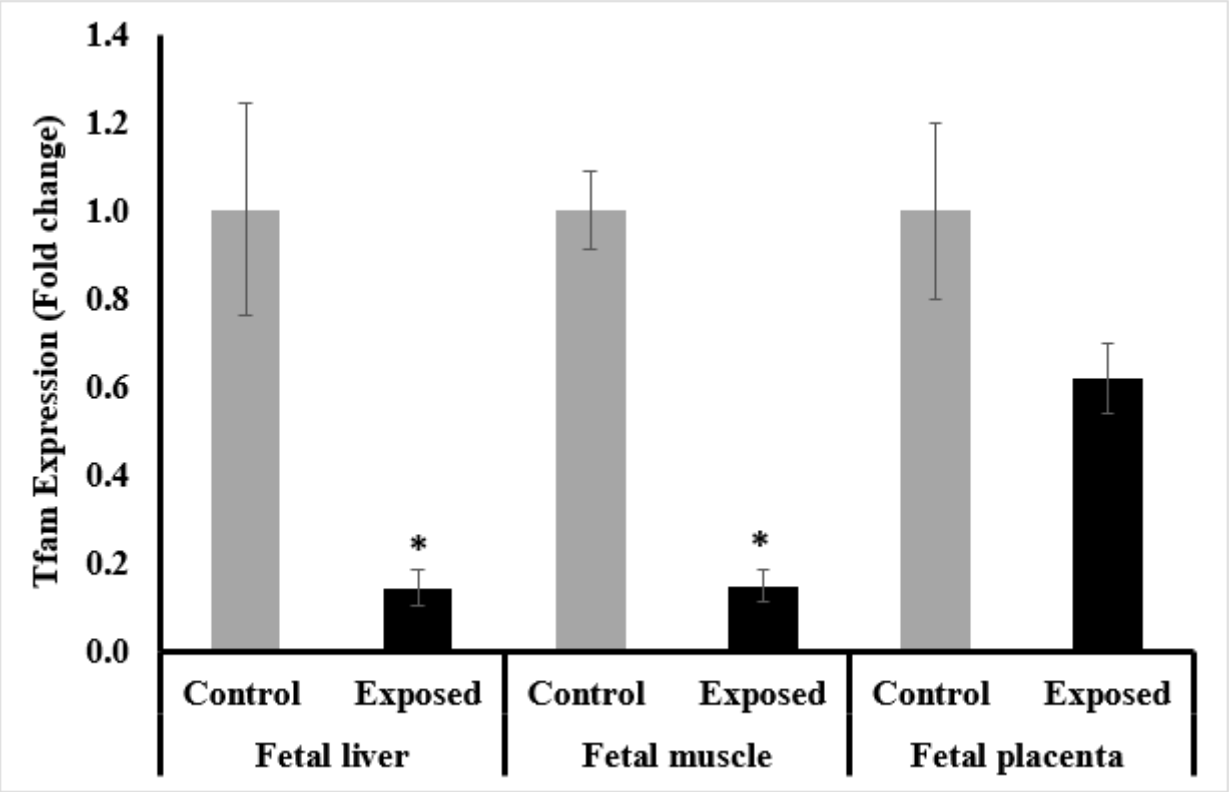

Figure 6

The change of Tfam gene expression (Fold change) in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. Data were illustrated as Mean ± SE Number of fetuses in each group = 10 *p < 0.05, indicating a statistically significant difference when compared with control group using independent sample t-test. Tfam: mitochondrial transcription factor A

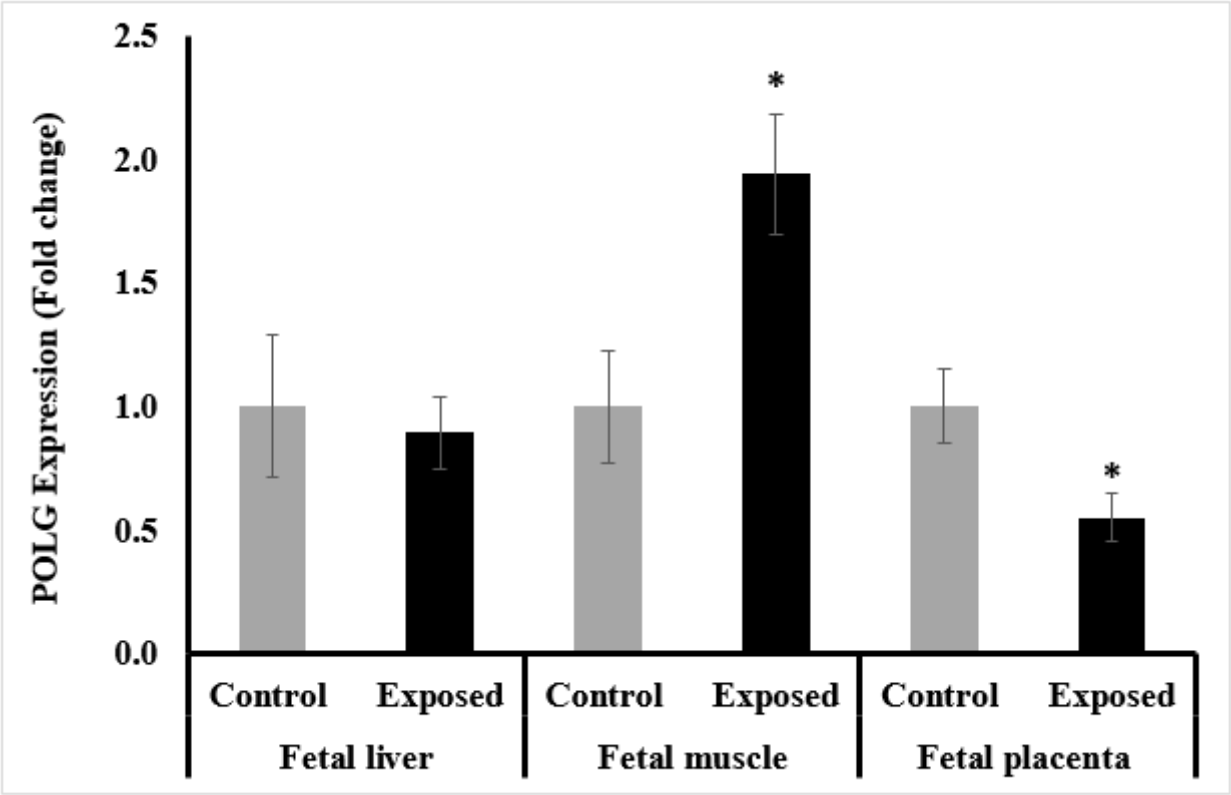

Figure 7

The change of POLG gene expression (Fold change) in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. Data were illustrated as Mean ± SE Number of fetuses in each group = 10 *p < 0.05, indicating a statistically significant difference when compared with control group using independent sample t-test. POLG: DNA polymerase gamma

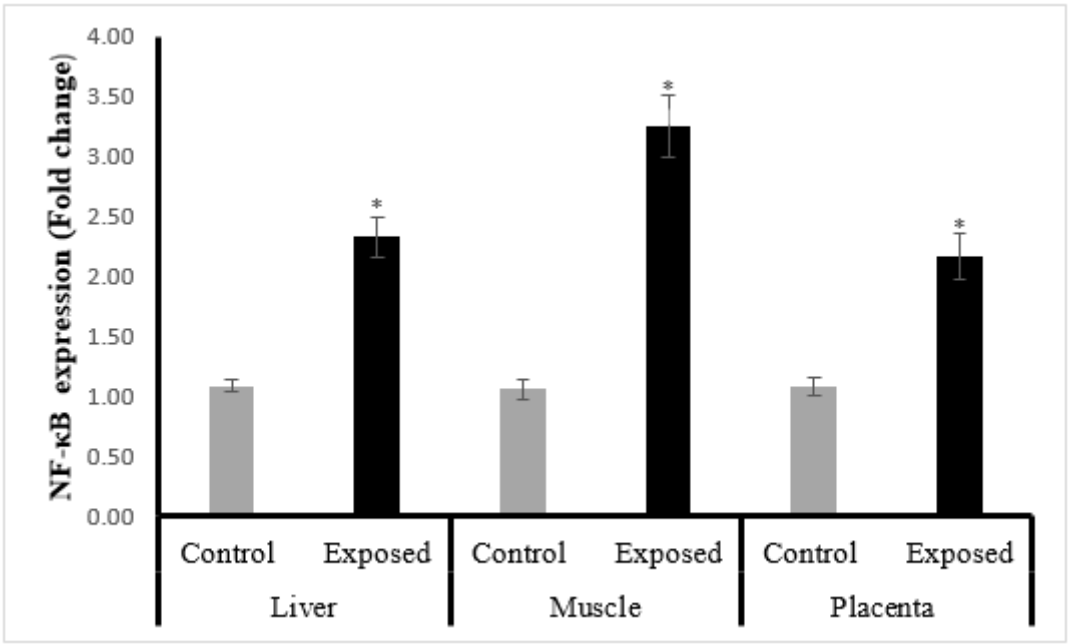

Figure 8

The change of NF-κB gene expression (Fold change) in fetal liver, skeletal muscle and placental tissues of fetuses of control and Sofosbuvir-exposed mothers. *p < 0.05, indicating a statistically significant difference when compared with the control group using independent sample t-test. NF-κB: nuclear factor kabba B

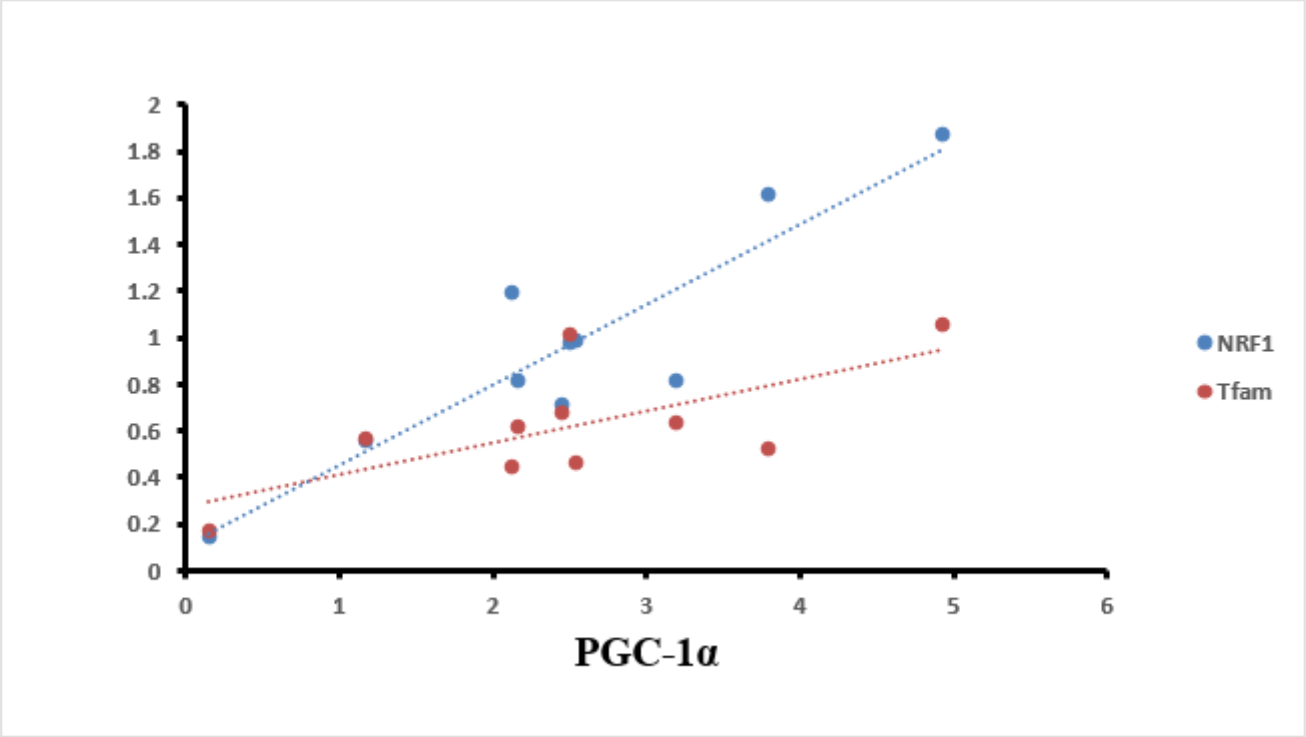

Figure 9

Correlation curve between PGC-1α expression with NRF-1 and Tfam expression in placental tissue of fetuses of Sofosbuvir-exposed mothers