Exercise-induced *Pgc-1α* Expression does not Lower mtDNA Point Mutation Levels but Downregulates *Tyrosine hydroxylase* Expression in the Striatum

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Abstract

Recent studies have highlighted evidence that somatic mtDNA point mutations can contribute to a premature aging phenotype in mice, including alopecia and sarcopenia. Interestingly, exercise has also been found to attenuate this premature aging phenotype, possibly through inducing the expression of *Pgc-1α*, a transcriptional coactivator that promotes mitochondrial biogenesis, in certain tissues such as muscle. The mechanisms of protection in the brain conferred by physical exercise are unknown. Here, through the use of the *Polg* mutator mouse model which has error-prone mtDNA replication, we show that although exercise has been reported to have neuroprotective effects, both endurance and voluntary exercise regimens did not lead to decreased mtDNA point mutation levels in the striatum of *Polg* mice. Furthermore, there was a trend towards decreased PGC-1α mRNA levels in exercised *Polg* mice, though the same samples revealed elevated PGC-1α protein levels, suggesting the presence of a negative feedback loop. Lastly, in order to gauge the function of dopaminergic neurons, tyrosine hydroxylase mRNA and protein levels were measured. Both were lowered in exercised *Polg* mice, suggesting a negative correlation between PGC-1α protein levels and *Th* expression.

Summary

Mitochondria have long been known as the cellular powerhouses as they act as sites for cellular respiration. However, recent research strongly suggests that mitochondria may contribute to the aging process, since somatic mitochondrial DNA (mtDNA) point mutations have been shown to result in accelerated aging. In addition, exercise has also been correlated with a lower likelihood of developing premature aging disorders of the brain, such as Parkinson's Disease (PD). In light of these findings, our study was carried out to determine if exercise could delay the process of premature aging in *Polg* mutator mice, and if so, to elucidate the underlying mechanisms. Premature aging was studied with reference to several markers of neuronal health, such as levels of Tyrosine Hydroxylase (TH), an enzyme that is crucial for dopamine synthesis. Our study has shown that while exercise has been touted to have neuroprotective effects, data pointing to possible underlying mechanisms remain elusive. Indeed, further research is required to validate the results gathered in our study.

1 Introduction

Despite the rapid pace at which research is taking place, many basic questions about mitochondrial biology remain unanswered. Well known as the cellular powerhouses that produce energy for cellular processes, the role of mitochondria in senescence and aging has only been recently discovered. Mitochondrial DNA (mtDNA), unlike nuclear DNA, consist of closed circular molecules that exhibit heteroplasmy. In other words, a mixed population of mtDNA molecules that differ in their base pair sequences exist within the same tissues, and even within individual cells[1]. In addition to the heteroplasmic nature of mtDNA, mtDNA also exhibits high copy numbers with up to 5000 copies of mtDNA in a single cell.

1.1 The Biological Role of mtDNA

mtDNA consist of 37 genes, all of which are crucial for normal mitochondrial function. Specifically, there are 13 protein coding genes, each coding for a component of the electron transport chain (ETC) which plays a crucial role in cellular respiration. In addition, the other genes consist of tRNA and rRNA genes, both of which are necessary for the production of proteins in the ETC[2].

1.2 mtDNA Mutations and Aging

mtDNA mutations comprise two categories: Single-base substitutions and deletions. Recently, a research paper suggesting the role of mtDNA deletions in driving the aging process sparked debate on the relative importance between base substitutions (also known as point mutations) and deletions in aging. Although both categories of mutations seem to be correlated with senescence[3], the relative abundance of point mutations (with a ratio of 1000 point mutations to every deletion) suggests that point mutations may be more closely associated with aging[4]. This is due to the high copy number of mtDNA, which favours, if not necessitates,

a large number of mutations before premature aging phenotypes can be overtly exhibited.

Bearing this in mind, it is likely that mutations accumulate through clonal expansion, whereby infidelity in DNA replication randomly amplifies the number of mtDNA mutations present. In fact, the abovementioned mechanism involving the loss of integrity in DNA replication corresponds with premature aging[5] and the accumulation of somatic mutations since these mutations are randomly generated.

1.3 Exercise and Aging

While it has been postulated for a long time that exercise slows the aging process, recent research has provided empirical results that support this claim. A study by *Tarnopolsky et al*[6] revealed that 5 months of endurance exercise in *Polg* mice induced systemic mitochondrial biogenesis and attenuated the aging process, as exercised *Polg* mice experienced a less drastic decline in brain weight as they aged in comparison to sedentary *Polg* mice. *Polg* mice are genetically modified mice that harbour DNA polymerase γ deficient in proofreading activity, such that they have elevated error rates in DNA replication[5]. Although the exact mechanisms implicated in mitigating the aging process in *Polg* mice remain elusive, evidence suggests the involvement of PGC-1α, a transcriptional coactivator that regulates mitochondrial metabolism. Notably, in *Polg* mice that were subjected to endurance exercise, elevated expression levels of *Pgc-1*α were reported in skeletal muscle[6]. In addition, increased mitochondrial abundance was also detected, possibly due to the protective effects of PGC-1α in terms of mitigating oxidative stress[7].

Furthermore, sedentary *Polg* mice displayed multisystem degenerative pathologies[8, 9] and DNA fragmentation. Since DNA fragmentation is a hallmark of aging and is usually driven by mitochondrial dysfunction, the link between PGC-1α and mitochondrial biogenesis[9] suggests that PGC-1α may play a role in mitigating the aging process.

1.4 Project Aim

Other than the accumulation of mtDNA mutations in skeletal muscle as observed in *Polg* mice[6], mtDNA mutations can also be found in the brain. A recent study by *Simon et al* found that dopamine-rich neurons in the substantia nigra accumulated high levels of mtDNA mutations in the early stages of Parkinsons Disease (PD)[10], a progressive neurodegenerative disorder characterized by the loss of dopamine. Prior research also suggests that physical activity has neuroprotective effects and reduces the risk of developing PD[11]. Since high levels of mtDNA mutations are implicated in PD and neuronal degeneration seems to be attenuated by physical exercise, it may be possible to draw a correlation between the effects of physical exercise on mitigating the premature aging process and mtDNA point mutation levels. Hence, one of the aims of this study was to determine if physical exercise confers neuroprotection and if so, find out if the mechanism involves lowering mtDNA point mutation levels.

Furthermore, exercise-induced expression of *Pgc-1α* has been found to correlate with the attentuation of premature aging in skeletal muscle[6]. Recently published research has also highlighted plausible evidence of common pathways for PGC-1α in the brain and skeletal muscle through the production of a hormone called irisin[12]. In light of these findings, this study aims to determine if PGC-1α attenuates aging in the brain. The Simon lab has also previously found that levels of tyrosine hydroxylase (TH), an enzyme essential in dopamine synthesis, are decreased in the *Polg* mutator mice. In order to gauge the function of dopaminergic neurons, levels of TH in the brain were studied. Last but not least, dopamine transporter (DAT) mRNA levels were also quantified as DAT is a marker of dopaminergic terminals in the striatum. By corroborating DAT and TH mRNA levels, the effects of exercise on dopaminergic function can be better understood.

1.5 Hypothesis

Firstly, elevated somatic mtDNA point mutation levels are implicated both in general multi-organ degenerative pathologies and neurodegeneration. In addition, prior research has shown that exercise induces the expression of *Pgc-1α*, a transcriptional coactivator involved in mitochondrial metabolism and antioxidant activities. Furthermore, PGC-1α is predicted to attenuate potentially deleterious effects of mtDNA point mutations in both skeletal muscle and in the brain. Hence, it is hypothesized that exercised *Polg* mice will have higher PGC-1α expression levels than SED-*Polg* mice, and that increased PGC-1α expression will attenuate the decline in TH levels.

2 Materials and Methods

2.1 Mutator Mouse Models

The *Polg* mouse mutator model was a central focus of this project. In *Polg* mice, mitochondrial DNA Polymerase γ, the main DNA polymerase responsible for replicating mtDNA, was mutated and lacked proof-reading exonuclease activity. Whereas functioning polymerase γ results in high fidelity mtDNA replication with an error rate of 1 error/500 000 bp, the lack of proofreading activity in *Polg*-deficient homozygotes increases the mutation frequency by three to 11 times[13]. This then leads to the accumulation of large numbers of random point mutations. In this experiment, two categories of mice, WT and *Polg* mice, were used. Specifically, homozygous *Polg* mutant mice were subjected to forced endurance exercise (END-*Polg*), voluntary exercise (Vol-*Polg*) or were kept sedentary (SED-*Polg*), and our analyses of these mice were compared to results in WT sedentary control mice (SED-WT). Brain tissue from WT exercised mice was not available for these studies.

2.2 mtDNA Point Mutation Analysis

Sequenced mtDNA was analyzed for point mutations by comparison to a reference sequence (Mouce D-loop, 15341-15896) using LaserGene SeqMan Pro (DNASTAR). The mean number of point mutations per million bp was calculated for mice from the four experimental groups. Only samples with 10000 bp or more of sequencing data were considered for purposes of accuracy.

2.3 Tyrosine hydroxylase (TH) Protein, DNA and RNA Isolation with Trizol Reagent

The striatum was dissected from frozen mouse brains (Tarnopolsky Lab, 2007). A volume of 100μl of lysis buffer was added to 5mg tissue samples, which were then homogenized with TissueLyser LT (QIAGEN, 2003) for 3 min at 50Hz. Thereafter, phase separation was carried out with chloroform to facilitate the extraction of protein, DNA and RNA with Trizol reagent (Sigma Aldrich). RNA was extracted from the top aqueous layer by addition of 50μl of 2-propanol and centrifugation at 12000 x g. DNA was precipitated from the interphase by addition of 30μl of 100% ethanol and centrifugation at 2000 x g. Thereafter, the DNA pellet was resuspended in 150μl of 75% ethanol. The supernatant from centrifugation at 2000 x g was also removed for protein precipitation with 150μl of 2-propanol and centrifugation at 7500 x g. The protein pellet was left to dry at room temperature and dissolved in 1% SDS buffer.

2.4 Quantification of mRNA Levels

Real Time-quantitative PCR (qPCR) was performed (protocol from *Fluke et al*, 2011) to determine the relative levels of PGC-1α, Th and DAT mRNA. Reverse transcription was first performed on mRNA to yield cDNA, which was then amplified and quantified with

Real-Time PCR using ABI Prism (Applied Biosystems). Results were normalized against two housekeeping genes, namely, 18S and TBP. The abovementioned genes were chosen as they have the same levels of expression across all cell types.

2.5 Bicinchoninic Acid (BCA) Protein Assay

To determine the volume of protein solution needed for the loading of 20μg of protein sample per Western Blot well, a BCA assay was performed (protocol from Muma Lab, 2007). Each of the 12 samples were analyzed in triplicate in a 96-well plate. Six standard reference concentrations (0, 1, 2, 3, 5, 10μg/l) were also made using Bovine Serum Albumin(BSA) and 2mg/ml BCA Standard (BIORAD). A covered plate was placed on shaker for 30 sec before being transferred to a 37°C dry incubator for 30 min. Samples developed a purple colouration, the intensity of which depended on the protein concentration. The plate (uncovered) was then placed on an automatic plate reader (BIORAD 680) at 540 nm, and readings were used in preparing samples for loading.

2.6 Western Blot

A volume of 10ml of 15% resolving gel was prepared with 2.3ml double-distilled H_2O (dd.H_2O), 5.0ml 30% Acrylamide mix, 2.5ml 1.5M Tris buffer (pH 8.8), 100μl 10% SDS, 100μl 10% APS and 4μl TEMED. A volume of 5ml of stacking gel was prepared with 3.4ml dd.H_2O, 0.83ml 30% Acrylamide mix, 0.63 ml 1.0M Tris buffer (pH 6.8), 50μl 10% SDS, 50μl 10% APS and 5μl TEMED. Samples were loaded and gel electrophoresis was done for 1h at 200V. Precision Plus Protein Dual Colour Standards #161-0374 (BIORAD) were used for molecular weight standards. After gel electrophoresis, proteins were transferred to a PVDF membrane for 2h at 100V. Thereafter, anti-TH primary antibody probes were added and the membrane left to incubate overnight. Secondary antibody probing with HRP was then performed, and

radiography was done to determine the relative amounts of TH in samples from SED-WT, SED-*Polg*, END-*Polg* and Vol-*Polg* mice.

3 Results

3.1 mtDNA point mutation levels

As foreseen, SED-WT mice tended to have lower point mutation levels than SED-*Polg* mice, with 93 mutations/million bp as compared to 194 point mutations/million bp. However, contrary to previously reported data that endurance exercise decreased the levels of mtDNA point mutations[6], our study found a non-significant increase in mtDNA point mutations in exercised *Polg* mice. Vol-*Polg* and SED-*Polg* mice had the highest levels of mutations, with 312 and 306 point mutations/million bp respectively (Fig. 1).

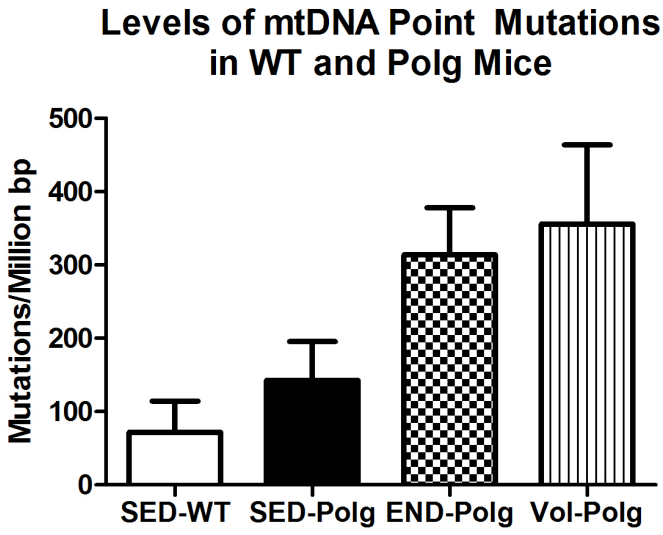

Figure 1: mtDNA Point Mutation levels in Striatum Samples. In accordance with data from previous studies[6], SED-WT mice exhibited lower occurrence rates of base substitutions in comparison with SED-*Polg* mice. Despite this, there was an unexpected trend towards increased mtDNA point mutation levels in exercised *Polg* mice when compared against SED-*Polg* mice.

3.2 *Pgc-1α*, *Th* and *Dat* Expression

When normalized against the 18S housekeeping gene, qPCR results revealed higher expression levels of *Pgc-1α* in SED-WT mice as compared to SED-*Polg* mice ($p < 0.05$). Interestingly, amongst the *Polg* groups, it was found that Vol-*Polg* mice had the lowest level of *Pgc-1α* expression ($p < 0.05$), followed by END-*Polg* mice ($p < 0.05$) and finally, SED-*Polg* mice, which exhibited the highest levels of PGC-1α expression. On the other hand, when PGC-1α levels were normalized to TBP, it was found that SED-*Polg* mice had higher PGC-1α expression levels than SED-WT mice, though this was not statistically significant. Also, when comparisons were made between the three *Polg* groups, the trend obtained from normalizing to 18S was reinforced, with Vol-*Polg* mice having the lowest PGC-1α expression levels, followed by END-*Polg* and SED-*Polg* mice (Fig. 2).

Furthermore, Western blot results for PGC-1α revealed the highest levels of PGC-1α in END-*Polg* mice, followed by Vol-*Polg*, SED-WT and SED-*Polg* mice (Fig. 3).

With respect to *Th* expression levels, *Polg* mice showed a non-significant increase in normalized TH mRNA as compared to WT mice. Amongst the three categories of *Polg* mice, END-*Polg* mice had the lowest *Th* expression levels while expression levels in Vol-*Polg* and SED-*Polg* mice were almost equivalent. Additionally, when normalized to TBP, it was found that SED-*Polg* mice had higher *Th* expression levels than SED-WT mice ($p < 0.05$). Also, amongst *Polg* mice, the trend of END-*Polg* mice having the lowest *Th* expression levels was reinforced by normalization with TBP, followed by SED-*Polg* and Vol-*Polg* in ascending order of *Th* expression levels (Fig. 4).

In order to find out if lower mRNA levels corresponded to lower TH protein levels, analysis of Western Blot results with band densitometry was performed. Results showed that END-*Polg* mice had the lowest TH protein levels and that the disparity in protein levels in SED-WT and END-*Polg* mice ($p < 0.05$) was statistically significant. Specifically, low TH levels in END-*Polg* mice were followed by that in Vol-*Polg*, SED-*Polg* and SED-WT

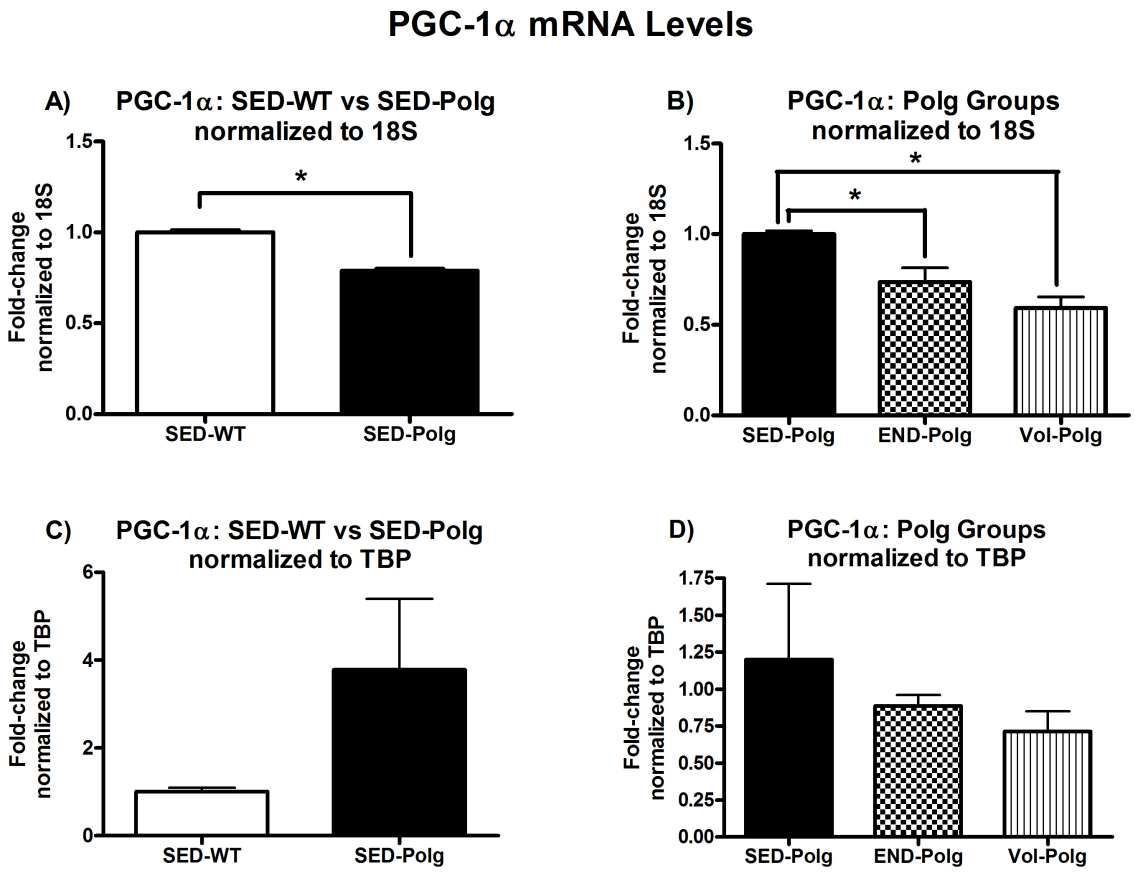

Figure 2: *Pgc-1α* mRNA levels. Despite the disparity when *Pgc-1α* levels were normalized against different housekeeping genes (A, C), statistically significant decreases in *Pgc-1α* levels were seen in SED-*Polg* mice (A). In addition, analysis with two-tailed t-tests amongst the *Polg* groups revealed lower *Pgc-1α* mRNA levels in exercised *Polg* mice (B).

mice in ascending order (Fig. 5). Taken into account together, the results run contrary to our hypothesis whereby it was postulated that brains of END-*Polg* mice would exhibit the highest Th protein levels. In addition, the results seem to suggest that exercise could decrease TH protein levels, whereas an increase in TH levels had been expected.

Last but not least, qPCR of DAT revealed a trend towards decreased *Dat* expression in *Polg* mice (Fig. 6). Moreover, there seems to be a non-significant trend towards increased *Dat*

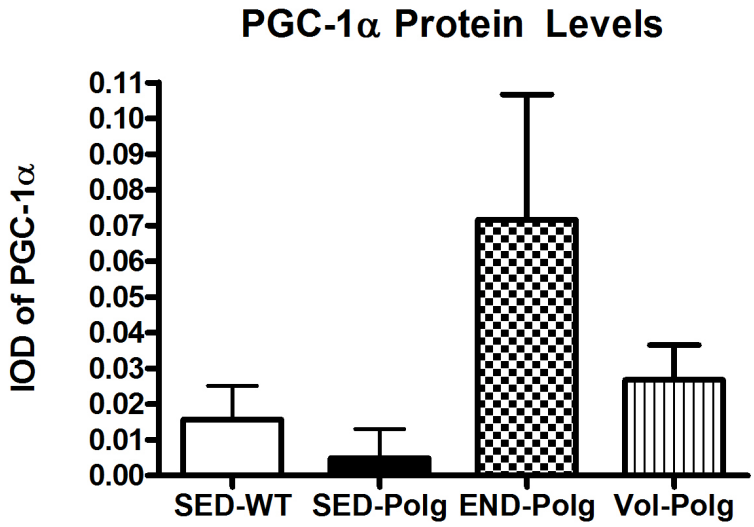

Figure 3: PGC-1α Protein Levels. Band densitometry revealed a trend towards higher PGC-1α protein levels in exercised *Polg* mice in comparison to SED-*Polg* mice, possibly highlighting an inverse correlation between PGC-1α mRNA and protein levels. This could suggest a negative feedback loop whereby high PGC-1α protein levels downregulated its own gene expression. "IOD" refers to "Integrated Optical Density" and is a measure of protein levels. The larger the IOD value, the higher the protein level.)

expression in exercised *Polg* mice in contrast with sedentary *Polg* mice. This was especially so for END-*Polg* mice, whereby *Dat* expression levels were approximately threefold of that seen in SED-*Polg* mice.

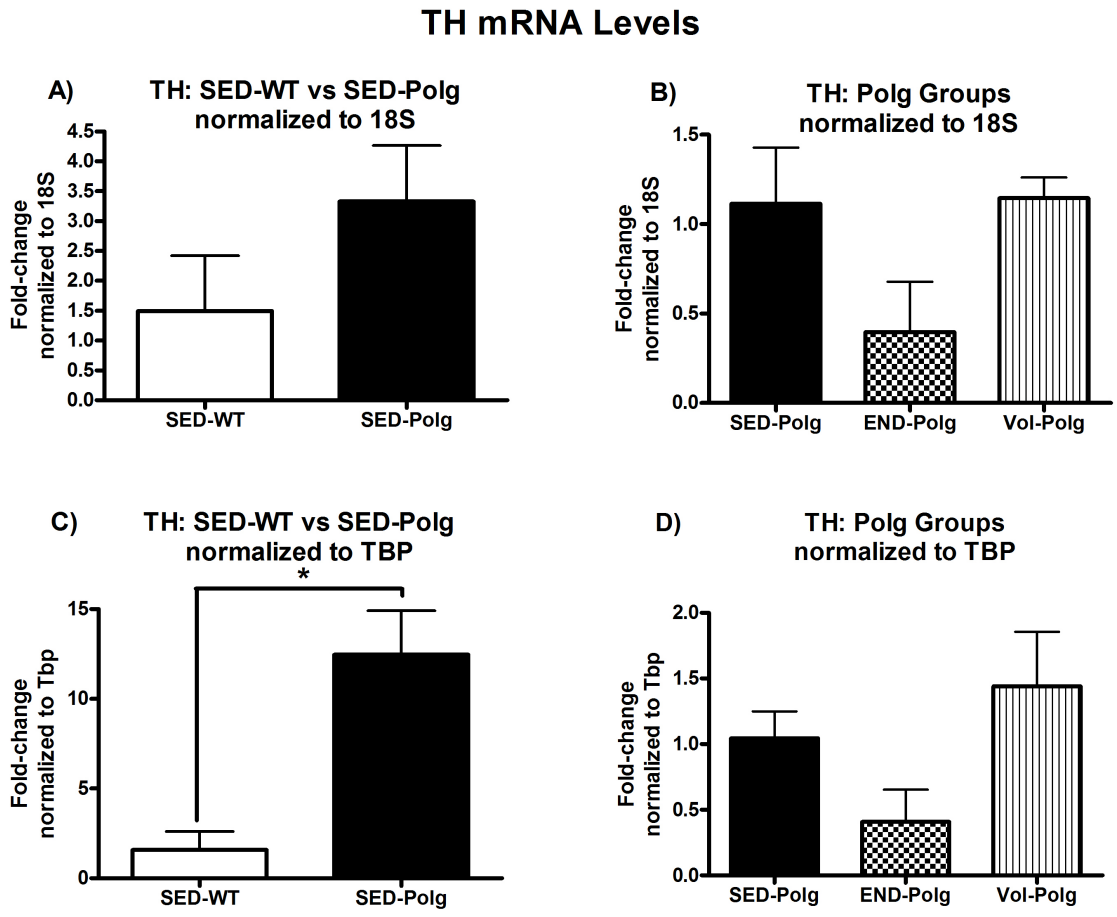

Figure 4: *Th* mRNA levels. SED-*Polg* mice were found to have higher TH mRNA levels than SED-WT mice when normalized to 18S (A) and TBP (C). Surprisingly, there was also a trend towards lowered TH mRNA levels in END-*Polg* mice in comparison with Vol-*Polg* and SED-Polg mice (B, D).

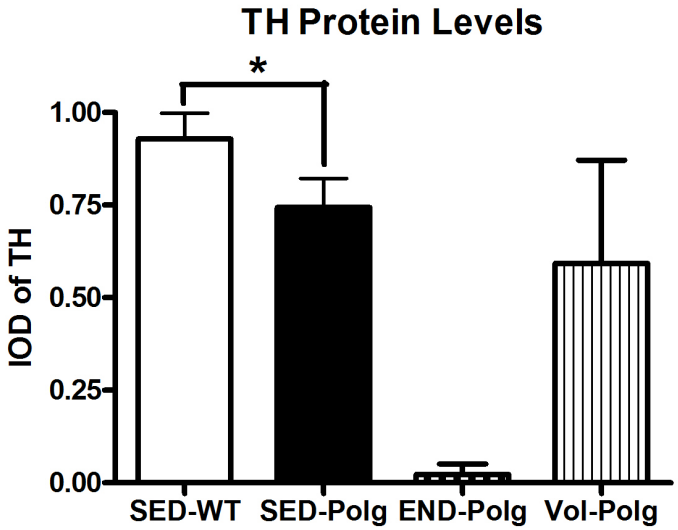

Figure 5: TH Protein levels. Protein levels mirror mRNA levels, whereby low TH protein levels corresponded to decreased TH mRNA levels in END-*Polg* mice. "IOD" refers to "Integrated Optical Density" and is a measure of protein levels. The larger the IOD value, the higher the protein level.

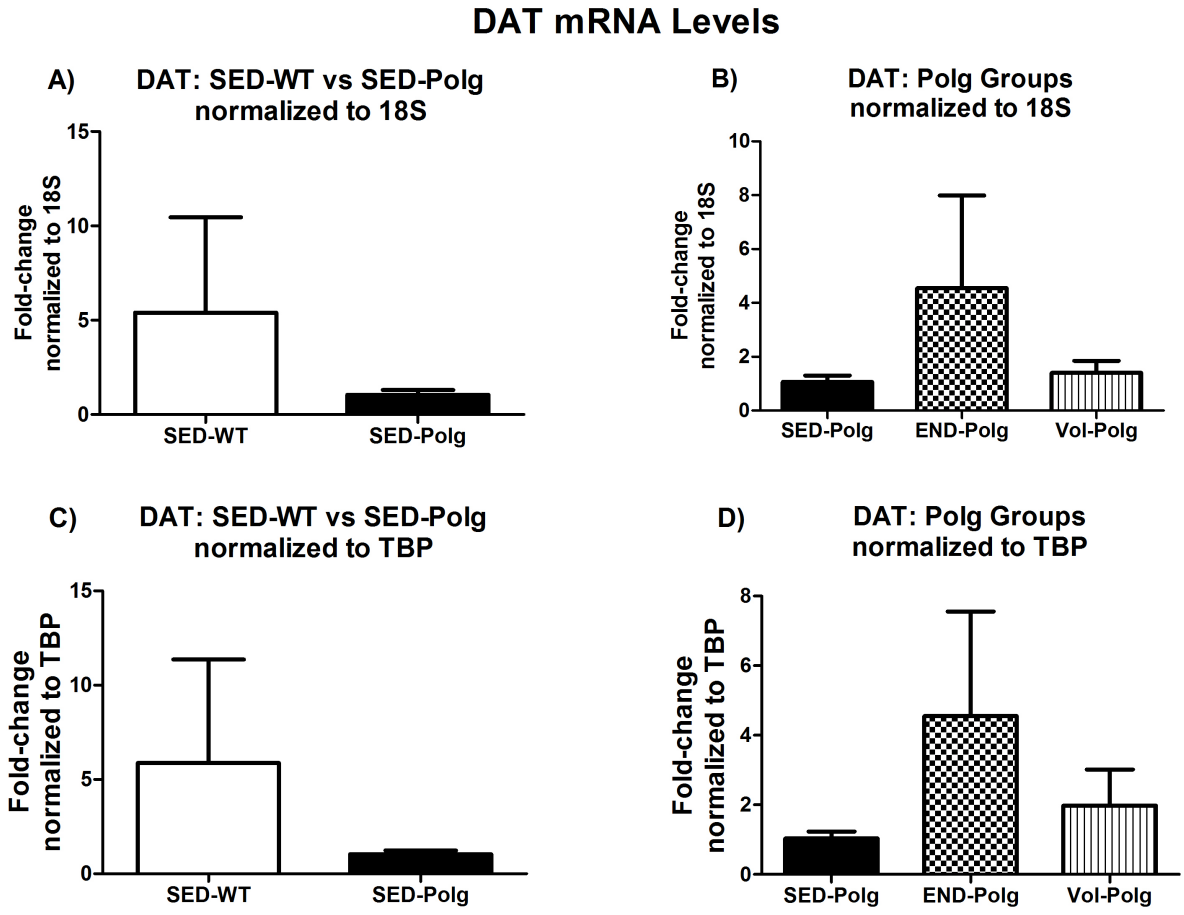

Figure 6: DAT mRNA levels. When normalized against both 18S and Tbp, SED-WT mice showed higher *Dat* expression levels (A, C). There was also a tendency towards increased *Dat* expression levels in exercised *Polg* mice in comparison to SED-*Polg* mice (B, D).

4 Discussion

With respect to mtDNA mutation levels, results seem to justify the use of *Polg* mutator mice as a model, since mtDNA mutation levels in SED-*Polg* mice were elevated when compared against that of SED-WT control mice. Despite this, Vol-*Polg* and END-*Polg* striatum samples surprisingly showed higher mtDNA point mutation frequency than that of SED-*Polg* mice. This could potentially be a result of increased oxidative stress induced by exercise[14] which resulted in increased production of free radicals and DNA damage that induced somatic mtDNA point mutations. This being said, exercise-induced oxidative stress has mostly been reported for acute exercise; endurance exercise, on the other hand, has been shown to raise levels of antioxidants and antioxidant enzymes, casting doubt on the hypothesis that elevated mtDNA mutation levels in exercised *Polg* mice was only due to exercise-induced oxidative stress.

In light of this, besides considering the possibility that exercise-induced oxidative stress contributed to elevated mtDNA point mutation levels, the abovementioned phenomenon might have been attributed to higher *Pgc-1α* expression levels. Studies have shown that *Polg* mice subjected to endurance exercise exhibit higher *Pgc-1α* expression levels[6] and consequently, increased mitochondrial biogenesis (i.e., higher rate of formation of new mitochondria). With increased mitochondrial biogenesis, the rate of mtDNA replication would be higher. This is likely to result in clonal expansion of mtDNA plasmids with point mutations and consequently, increased mutation levels in exercised *Polg* mice.

This hypothesis was tested by looking at Pgc-1α expression levels across the four experimental groups. When Pgc-1α was normalized against different housekeeping genes, there was some disparity in results, with SED-WT mice showing higher Pgc-1α expression levels than SED-*Polg* mice when normalized to 18S ($p < 0.05$) whereas the converse was true for Tbp. This could have resulted from slight inaccuracies due to a high standard error of the

mean (SEM) for Tbp in SED-*Polg* mice. Despite the slight discrepancies, consistently lower Pgc-1α expression levels were found in exercised *Polg* mice as opposed to SED-*Polg* mice. This could have been due to a negative feedback loop, whereby higher levels of PGC-1α protein in exercised mice led to a decrease in its own mRNA levels. To determine if this hypothesis was valid, a Western blot was performed to determine relative PGC-1α protein levels amongst the four groups of mice. Although further experimentation is required, preliminary PGC-1α Western Blot results seem to indicate that END-*Polg* mice had the highest PGC-1α protein levels, suggesting that PGC-1α protein downregulates its own expression at high concentrations.

Furthermore, in accordance with the aim of this project, i.e. to determine if exercise attenuates the decline in TH levels seen with premature aging in the *Polg* mice, TH mRNA and protein levels were measured. Interestingly, there was a trend towards lower *Th* expression in SED-WT compared to SED-*Polg* mice. In addition, exercised *Polg* mice also had lower expression levels than SED-*Polg* mice. This trend was reinforced by Western blot results, whereby analysis with band densitometry revealed decreased TH protein levels in exercised *Polg* mice in comparison to SED-*Polg* mice. Notably, studies of postmortem PD brain have shown increased somatic mtDNA point levels in the setting of reduced TH protein levels. Hence, it could be possible to account for this phenomenon by drawing parallels to the abovementioned PD studies. Specifically, increased oxidative stress in exercised *Polg* mice may have led to higher mtDNA point mutation levels, which corresponded to lower TH protein levels.

Taken together, when PGC-1α protein levels were juxtaposed against TH protein levels, results suggest an inverse correlation between levels of both proteins. Unpublished data from our lab suggests that downregulation of *Th* expression as seen in END-*Polg* mice could have been due to overexpression of *Pgc-1α* through downregulation of *Pitx3* expression. PITX3, a transcription factor, plays a critical role in the development of substantia nigra dopaminergic

neurons. Previous research has found that lowered *Pitx3* expression corresponded to lowered TH levels[15]. Furthermore, an independent study by the Simon Lab provided further evidence that increased PGC-1α levels led to lowered PITX3 mRNA and protein levels, which ultimately culminated in decreased TH levels. By applying the abovementioned findings to the data gathered in this study, it is plausible that higher PGC-1α levels in exercised *Polg* mice could have resulted in a decline in PITX3 levels, consequently leading to a reduction in TH levels. This being said, the results do not necessarily negate endurance exercise as a potential method for ameliorating the effects of PD progression, as PD patients tend to exhibit lower basal PGC-1α levels and endurance exercise may restore normal PGC-1α levels without much risk of inducing PGC-1α overexpression.

Last but not least, other than measuring *Th* expression, expression levels of *Dat*, a marker of dopaminergic terminals in the brain, were also quantified. In accordance with previous literature that mice carrying the *Polg* mutation have lowered DAT levels, a decline in *Dat* expression was observed in SED-*Polg* mice when benchmarked against SED-WT mice, and END-*Polg* mice exhibited the highest *Dat* expression levels amongst the *Polg* experimental groups. These results suggest that DAT was likely to have been downregulated in SED-*Polg* in comparison to SED-WT mice. Results gathered mirror those found in a study by *Joyce et al*, whereby it was shown that remaining neurons in postmortem PD brains exhibited elevated TH mRNA levels but had lower DAT mRNA levels[16]. This being said, the exact mechanisms behind the putative inverse correlation between TH and DAT mRNA levels remain unknown. Lastly, the trend towards increased DAT expression in exercised *Polg* mice reflects that exercise could promote neuroprotection by increasing DAT expression.

5 Limitations and Further Directions

Whilst this study has shown that exercise may not necessarily attenuate premature aging in terms of lessening the decline in TH protein levels, other parameters need to be studied to verify if exercise contributes to a slower rate of premature aging. Firstly, due to resource constraints, the WT group only consisted of samples from SED-WT mice. In order to make fairer comparisons between exercised and sedentary mice, future studies will include striatum samples from END-WT and Vol-WT mice. In addition, another parameter of interest is the mtDNA copy number, which would act as a gauge for mitochondrial abundance. Previous studies have established a positive correlation between mitochondrial biogenesis and the attenuation of premature aging in skeletal muscle[6]. Hence, in order to determine if exercise promotes mitochondrial biogenesis in the brain, the quantification of mtDNA copy number would be of interest for future studies.

Furthermore, as the reason behind elevated mtDNA point mutation levels in exercised *Polg* mice remains unclear, the hypothesis that exercise-induced oxidative damage contributes to this phenomenon can be validated by performing oxidative stress assays. This will provide a more definitive answer as to whether exercise-driven oxidative stress could account for increased mtDNA point mutation levels in exercised *Polg* mice. Finally, expression levels of non-dopaminergic synaptic terminal markers such as GABAergic and cholinergic markers will be studied. This will provide a more in-depth understanding of observed *Th* and *Dat* expression levels and test the hypothesis that decreased expression levels could have been a result of terminal death.

6 Conclusion

In the final analysis, this study has found that exercise, though thought to have neuroprotective effects in PD, do not necessarily lower somatic mtDNA point mutations. Instead,

non-significant increases in mtDNA base substitutions were found for exercised *Polg* mice, possibly due to increased mitochondrial biogenesis as a result of exercise-induced *Pgc-1α* expression or due to increased oxidative stress. Lowered levels of PGC-1α mRNA were also detected in exercised *Polg* mice, though this was in contrast to elevated PGC-1α protein levels observed in the same samples, possibly suggesting that PGC-1α protein regulated its own expression levels in a negative feedback loop. Notably, lowered TH mRNA and protein levels were observed in exercised-*Polg* mice and corresponded to increased PGC-1α protein levels. Two reasons that could possibly account for these results have been put forth, the first being that decreased TH mRNA and protein levels could have been due to exercise-induced oxidative stress. Alternatively, results observed may be attributed to a mechanism found in an unpublished study by the Simon Lab that *Pgc-1α* overexpression downregulates *Th* expression. Despite the preliminary nature of these findings, the possibilities they raise continue to be of interest for future studies, for understanding the molecular mechanisms and correlation between exercise and premature aging is likely to play an integral part in the development of novel therapeutic interventions for targeting premature aging.

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