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ORIGINAL ARTICLE

Lower Plasma Selenoprotein P Levels in Regularly Exercising Young Adults

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Abstract

Objective. Physical exercise can provide many health benefits in humans. Exercise-induced reactive oxygen species (ROS) formation and its downstream signaling cascades are reported to induce mitochondrial biogenesis in exercising tissues. Selenoprotein P (SELENOP) is the antioxidant hepatokine whose hypersecretion is associated with various metabolic diseases. It was reported to impair exercise-induced reactive oxygen species signaling and inhibit subsequent mitochondrial biogenesis in mice. However, the relationship between selenoprotein P and mitochondrial dynamics in humans has not yet been reported. While reduction of plasma selenoprotein P becomes an attractive therapeutic target for metabolic diseases, the role of regular exercise in this regard is still unknown. This study aimed to analyze the influence of regular habitual exercise on plasma selenoprotein P levels and its association with leucocyte mitochondrial DNA copy number in healthy young adults.

Methodology. Plasma selenoprotein P levels and leucocyte mitochondrial DNA copy numbers were compared in 44 regularly exercising subjects and 44 non-exercising controls, and the correlation between the two parameters was analyzed. Plasma selenoprotein P levels were measured by Enzyme-linked Immunosorbent Assay, and leucocyte mitochondrial DNA copy numbers were measured using the qPCR method.

Results. The regular-exercise group had lower plasma selenoprotein P levels with higher leucocyte mitochondrial DNA copy numbers than the non-exercise group. There was a tendency of negative correlation between the two variables in our studied population.

Conclusion. Regular habitual exercise has a beneficial effect on reducing plasma selenoprotein P levels while raising mitochondrial DNA copy numbers.

Key words: mitochondria, physical exercise, reactive oxygen species, selenoprotein P

INTRODUCTION

It is undisputedly accepted that physical exercise can provide many health benefits in humans beyond body fitness.[1,2] During physical exercise, numerous metabolic adaptations in various tissues occur to meet the increased oxidative capacity and metabolic demands of the exercising tissues.[3-5] One major event during physical exercise is the transient induction of sub-pathological amounts of reactive oxygen species (ROS),[6] mainly generated from the mitochondrial respiratory chain as a byproduct of accelerated cellular respiration during physical exercise.[7] Researchers are gradually realizing that exercise-induced ROS plays a crucial role in proper cellular functioning by acting as intracellular messengers in various signaling cascades.[8,9]

Previous studies reported that the production of ROS in skeletal muscle during prolonged endurance exercise plays an important role in hormesis-like adaptive changes in skeletal muscle. In particular, acute exposure to ROS during physical exercise can activate the 5' adenosine monophosphate-activated protein kinase - peroxisome proliferator-activated receptor gamma (AMPK-PGC-1α) signaling cascade,[9,10] which plays a central role in mitochondrial biogenesis and mitochondrial DNA maintenance.[11,12] Furthermore, exercise-induced ROS signaling induces the expression of endogenous antioxidant enzymes including manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx) through the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), to restore cellular redox homeostasis.[13] Those adaptive changes are important for the health-promoting effects of regular exercise, supporting the fact that exercise is an antioxidant and medicine.

Selenoprotein P (SELENOP), encoded by the *SELENOP* gene in humans, is a selenium transport protein mainly

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secreted by the liver.[14] It is reported to have an antioxidant capacity through direct enzymatic action or by supplying selenium to synthesize intracellular antioxidant enzymes.[15] Notably, one recent report indicated that SELENOP deficiency in mice increased exercise-induced ROS formation and subsequent AMPK signaling cascade with higher mitochondrial DNA content in skeletal muscle.[16] In their study, they also reported over-activity of SELENOP impaired hydrogen peroxide (H_2O_2) -induced AMPK phosphorylation and mitochondrial biogenesis in the myocyte, indicating the inhibitory action of SELENOP on mitochondrial biogenesis.[16] However, the relationship between SELENOP and mitochondrial dynamic in human studies has not been reported.

Previously, hepatic overproduction of SELENOP was reported to be involved in insulin resistance and hyperglycemia in patients with type 2 diabetes,[17] hypoadiponectinemia[18] and impaired angiogenesis by vascular endothelial growth factor (VEGF) resistance.[19] Misu et al., reported that overproduction of SELENOP impairs insulin signaling and dysregulates cellular glucose metabolism by reducing insulin-stimulated insulin receptor phosphorylation and subsequent Akt phosphorylation in hepatocytes and glucose uptake into myocytes.[19] Since the reduction of plasma SELENOP has been considered the potential target for the prevention and treatment of metabolic diseases, exploring various factors which influence plasma SELENOP levels has interested many researchers. One potential candidate is regular exercise, which has been reported to have beneficial effects in preventing various metabolic diseases.[1] To our best knowledge, there have been no previous reports about the influence of regular exercise on plasma SELENOP levels in the healthy young population.

Therefore, the general objective of this study was to analyze the influence of regular exercise on plasma SELENOP levels and determine whether there is a correlation between plasma SELENOP levels and leucocyte mitochondrial DNA copy numbers (mtDNA CN) in young adults.

The specific objectives were to (1) Measure plasma SELENOP levels and mtDNA CN in regularly exercising and non-exercising healthy young adults; (2) Compare plasma SELENOP levels and mtDNA CN between regularly exercising and non-exercising healthy young adults, and (3) Assess the correlation between the two parameters in our studied population. This study measured the leucocyte mtDNA CN as an indicator of mitochondrial biogenesis and mitochondrial abundance in the cell.[20]

METHODOLOGY

Study design and participants

This study was a cross-sectional, comparative study performed on 44 male student volunteers from the University of Medicine 2 (UM2), Yangon, Myanmar, and 44 male students from the Institute of Sports and Physical Education (ISPE), Yangon, Myanmar.

Sample size was calculated based on the previous study that measured serum selenium levels in non-exercising and regularly-exercising groups,[21] as serum selenium levels were reported to have a strong and significant correlation with circulating SELENOP levels.[22,23] With 95% confidence interval and 90% power of the study, the calculated required sample size for each group was 44 and the total sample size was 88.

We defined a regularly-exercising person as one who does moderate intensity endurance and/or resistance exercises for a minimum of 300 minutes per week for more than 6 months.[24,25] Forty-four healthy subjects from ISPE, Yangon, Myanmar who met the criteria participated in the regular-exercise group. In contrast, the non-exercise control group comprised volunteer medical students from UM2, Yangon, Myanmar with no history of regular exercise within one year before the study. All the participants were non-obese, non-diabetic, normotensive, apparently healthy young males between the ages of 16 to 20 years. Individuals with a previous history of diabetes, hypertension, liver diseases or currently taking selenium or antioxidant vitamin supplementation were excluded from the study. Our study was approved by the Institutional Review Board, Department of Medical Research Myanmar (Ethics/DMR/2020/026). All participants were volunteers who provided written informed consent for participation.

Physical examination and blood sample collection

Personal data collection, history taking, anthropometric assessment and blood pressure measurement were performed before taking the fasting blood sample. Venous blood samples were put in EDTA tubes and centrifuged at 2500×g for 10 minutes for buffy coat and plasma separation. According to the manufacturer's instructions, genomic DNA isolation was performed from the buffy coat samples on the same day of sample collection using the blood DNA mini kit (Invitrogen, USA). During DNA extraction, the lysate of the buffy coat layer was treated with RNAase solution to eliminate possible RNA contamination in the purified DNA samples.

Measurement of plasma SELENOP levels

The SELENOP levels of the plasma samples were measured via the Enzyme-linked Immunosorbent Assay (ELISA) method by using the Human selenoprotein P (SELENOP) ELISA kit (Catalogue No: abx251264, Abbexa, UK) according to the manufacturer's instructions.

Measurement of leucocyte mitochondrial DNA copy number

DNA concentration of each sample was measured and diluted by nuclease-free water to prepare a 10ng/µl con-

centration. After that, mtDNA CN in each sample was determined by quantitative PCR analysis of mitochondrial *ND1* gene and normalized by simultaneous measurement of the nuclear gene, β-globulin (*HBB*) in QuantStudio™ 3 Real-time PCR system using SYBR™ Green PCR Master Mix (Thermo Fisher Scientific, USA). The forward primer 5′- CCC TAA AAC CCG CCA CAT CT-3′ and reverse primer 5′- GAG CGA TGG TGA GAG CTA AGG T-3′ were used for mitochondrial *ND1* gene analysis. For the nuclear β-globulin (*HBB*) gene, the forward- 5′- GCT CGG TGC CTT TAG TGA TG- 3′ and reverse- 5′- AAA ACA TCA AGC GTC CCA TAG AC- 3' primer set was used. PCR reaction was performed twice with two sets of forward and reverse primers for the mitochondrial *ND1* and nuclear *HBB* gene.

After denaturation at 95°C for 10 minutes, the samples were subjected to 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The threshold cycle number (Ct) values were defined as the numbers of PCR cycles required to produce a 20ng DNA product. The Ct values of the *ND1* and nuclear *HBB* gene were determined for each sample. The relative mitochondrial copy number in each sample was calculated by the Relative copy number (Rc) = $2^{-\Delta Ct}$, where ΔCt is the Ct^{HBB}- Ct^{ND1} as described previously[25]. After which, fold changes in mtDNA CN in each sample were calculated by setting the mean for the control group as 1.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, Version 20.0. Data were expressed as mean ± standard deviation. The normal distribution of each parameter was checked by the Shapiro-Wilk test in SPSS. The student's *t*-test for parameters with a normal distribution (BMI, height, weight, and plasma SELENOP) and Mann-Whitney U test for non-normal data (Age, fasting blood sugar (FBS), systolic blood pressure, diastolic blood pressure, and mtDNA CN) respectively, were used to analyze statistically significant differences between regular-exercise and non-exercise groups. A *p*-value of <0.05 was set as the level of significance. Correlations between different variables were analyzed using Pearson's correlation coefficient.

RESULTS

The comparison of biochemical parameters between the non-exercise and regular-exercise groups are described in Table 1. The non-exercise group was found to be significantly older than the regular-exercise group ($p<0.001$), whereas no significant difference was found for height, weight, body mass index (BMI), systolic, or diastolic blood pressures. Notably, the regular-exercise group was found to have significantly lower FBS levels than the non-exercise group ($p=0.001$).

Regularly exercising subjects had lower plasma SELENOP levels and higher mtDNA CN than non-exercising counterparts

As shown in Table 1, mean plasma SELENOP levels of the regular-exercise group (3.70 ± 0.80 μg/ml) was found to be significantly lower than the non-exercise group (4.63 ± 1. 30 μg/ml) ($p < 0.001$) (Table 1 and Figure 1A). Mitochondrial DNA copy number (mtDNA CN) was also found to be significantly higher in the regular-exercise group than the non-exercise group ($p < 0.001$) (Table 1 and Figure 1B).

To exclude the effect of age differences between the two groups, the subjects were initially stratified into two age groups (<18 years and ≥18 years), and the parameters were then compared in each group. As shown in Table 2,

Table 1. Comparison of biochemical parameters of participants

Parameter	Non-exercise group (N = 44)	Regular-exercise group (N= 44)	*p* value
Completed age (years)	18.27 ± 1.45	16.89 ± 0.75	< 0.001***
Height (m)	1.68 ± 0.06	1.71 + 0.05	0.017*
Weight (kg)	60. 13 ± 10. 80	61.89 ± 5.37	0.311
Body mass index (kg/m^2)	21.09 ± 2.72	21.06 ± 1.60	0.941
Systolic blood pressure (mmHg)	114.55 ± 9.51	115.57 ± 7.17	0.570
Diastolic blood pressure (mmHg)	74.66 ± 8.03	74.00 ± 7.31	0.932
Fasting blood sugar (mg/dl)	103.70 ± 9.41	93.61 ± 13.78	0.001**
Plasma SELENOP (μg/ml)	4.63 ± 1. 30	3.70 ± 0.80	< 0.001***
mtDNA CN	1 ± 0.61	2.44 ± 0.81	< 0.001***

Results are expressed as mean ± standard deviation. p value was calculated by either Student's unpaired t-test or Mann-Whitney U test for normally distributed and non-normally distributed parameters respectively. *p <0.05, **p <0.01 and ***p <0.001.
Abbreviation; mtDNA CN = mitochondrial DNA copy numbers, SELENOP = Selenoprotein P

Table 2. Comparison of plasma SELENOP and mtDNA CN between non-exercise and regular-exercise groups stratified by age

	Age <18 years			Age ≥18 years		
	Non-exercise group (N = 15)	Regular-exercise group (N = 34)	*p*	Non-exercise group (N = 29)	Regular-exercise group (N = 10)	*p*
Plasma SELENOP (ug/ml)	4.76 ± 1.36	3.60 ± 0.70	<0.001***	4.57 ± 1.28	3.67 ± 1.01	0.049*
mtDNA CN	1.00 ± 0.66	2.35 ± 0.82	<0.001***	1.00 ± 0.60	2.76 ± 0.72	<0.001***

Results are expressed as mean ± standard deviation. p-value was calculated by Student's unpaired t-test for plasma SELENOP levels and Mann-Whitney U test for mtDNA CN respectively. *p<0.05, **p<0.01 and ***p<0.001.
Abbreviation: mtDNA CN = mitochondrial DNA copy numbers, SELENOP = Selenoprotein P

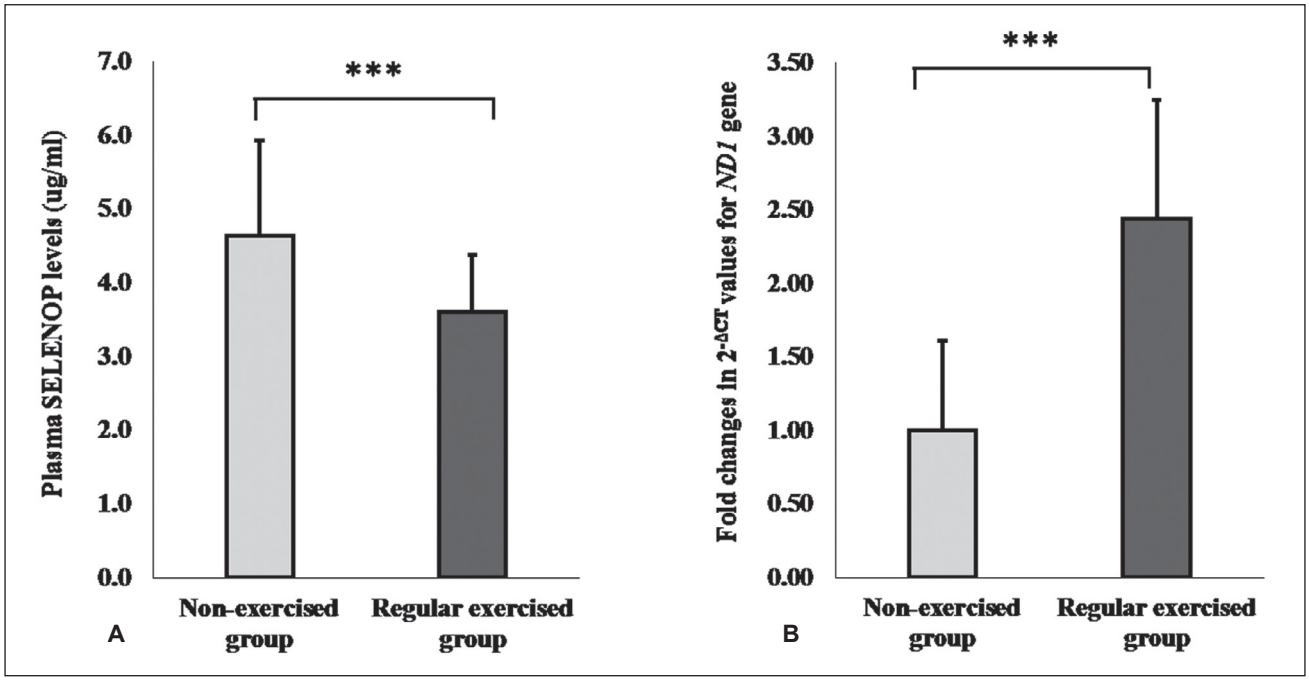

Figure 1. Differences in **(A)** plasma SELENOP levels and **(B)** mtDNA CN between non-exercise and regular-exercise groups (N= 44 for each group). mtDNA CN was calculated by $2^{-\Delta CT}$ values of mitochondrial ND1 gene copy number normalized to nuclear HBB gene. Fold changes in $2^{-\Delta CT}$ were compared between the non-exercise group and regular-exercise group. Data are presented with mean ± SD.

***p<0.001 by Student's unpaired t-test for plasma SELENOP levels and Mann-Whitney U test for mtDNA CN, respectively.

the regular-exercise group had significantly lower plasma SELENOP with higher mtDNA CN than the non-exercise controls in both age strata. Therefore, our findings confirmed that the age difference between non-exercise and regular-exercise groups did not affect the statistically significant difference in plasma SELENOP levels and mtDNA CN between the two studied groups.

Correlation between plasma SELENOP levels and mtDNA CN

We then analyzed the bivariate correlations between plasma SELENOP levels and various biochemical parameters in our subjects. As shown in Table 3, plasma SELENOP levels showed a significant, weak positive correlation with age (R=0.288, p=0.006) and BMI (R=0.273, p=0.01), while no correlation was detected between plasma SELENOP and FBS (R=0.058, p=0.59), height (R=0.016, p=0.884), systolic and diastolic blood pressures (R=0.057, p=0.599 and R=0.055, p=0.614 respectively). A weak, positive but nonsignificant correlation was found between plasma SELENOP level and weight (R=0.203, p=0.058). Notably, there was a trend of negative correlation between plasma SELENOP levels and mtDNA CN (R=- 0.203), although the p-value failed to reach a statistically significant level (p=0.059).

Table 3. Bivariate correlation between plasma SELENOP levels and various metabolic parameters in the studied population (N= 88)

	Plasma SELENOP
Fasting blood sugar	R = 0.058, p = 0.590
Age	R = 0.288, p = 0.006**
BMI	R = 0.273, p = 0.01*
Height	R = 0.016, p = 0.884
Weight	R = 0.203, p = 0.058
Systolic blood pressure	R = - 0.057, p = 0.599
Diastolic blood pressure	R = 0.055, p = 0.614
mtDNA CN	R = - 0.203, p = 0.059

Statistical method by Pearson's correlation coefficient. *p< 0.05, **p< 0.01 and ***p< 0.001. Abbreviation: BMI = Body Mass Index, mtDNA CN = mitochondrial DNA copy numbers, SELENOP = Selenoprotein P

DISCUSSION

Selenoprotein P (SELENOP) is the major selenium transport hepatokine. Its hepatic hypersecretion was previously reported to be associated with various metabolic disorders.[16-19,21,26,27] In this regard, exploring various factors which can influence circulating SELENOP has interested researchers aiming for a more comprehensive management of metabolic diseases.

To examine whether regular exercise has benefits in reducing circulating SELENOP levels, we compared plasma SELENOP levels in non-exercising controls and regularly-exercising young adults. All the participants were relatively healthy male students between the ages of 16 to 20 years. The two groups were comparable in height, weight, BMI, systolic and diastolic blood pressures. The only significant difference was that fasting blood glucose levels were lower in the regular-exercise group, consistent with the well-known beneficial effects of physical exercise on glucose metabolism.[28-30]

In this study, we also found that plasma SELENOP levels were significantly lower in regularly-exercising subjects compared to their non-exercising counterparts, independent of age.

To our best knowledge, this is the first report of the influence of long-term regular exercise on plasma SELENOP levels in healthy individuals. It was previously reported that plasma SELENOP levels did not change significantly after eight weeks of aerobic exercise training in sedentary postmenopausal women[16] or after an acute bout of 60 minutes of moderate-intensity treadmill training in obese men.[31] In contrast, the regularly exercising participants in this study were healthy young athletes who had been getting regular sports-type exercise training for approximately 20.06 ± 5.04 hours per week for more than two years. The training program included at least 3 hours per day, six days a week of endurance exercise such as running and jogging in addition to sports type-specific training for each student. Therefore, the type, duration and intensity of physical exercise may be the critical factors in exercise-mediated suppression of plasma SELENOP levels.

A previous study reported that plasma SELENOP levels were higher in patients with pulmonary arterial hypertension (PAH) compared with controls, and higher plasma SELENOP level was associated with poor outcomes in PAH patients.[32] Moreover, higher plasma SELENOP levels were reported to be positively associated with carotid intima-media thickness and increased risk of heart failure.[33,34] As our study found lower plasma SELENOP levels in regular exercise, it is possible that the cardio-protective benefits of regular exercise may at least partly be mediated by its action on the reduction of plasma SELENOP levels.

There are some potential mechanisms for lower plasma SELENOP levels in physical exercise. First, since the liver is the primary source of circulating SELENOP,[35] the lower plasma SELENOP levels in regularly exercising persons may be due to lower hepatic *SELENOP* expression. Takayama et al[36] reported that hepatic AMPK activation by metformin decreased nuclear localization and subsequent transcriptional inactivation of FoXO3, resulting in suppression of hepatic SELENOP expression. As physical exercise has been reported to enhance hepatic AMPK activities,[37,38] exercise-induced AMPK-FoXO3 activation may be responsible for suppressing hepatic *SELENOP* expression and subsequent lower plasma SELENOP levels in physical exercise. More longitudinal and in-vitro experiments are in great demand to confirm the assumption.

Second, the lower SELENOP levels in the regular-exercise group may be related to accelerated uptake and cellular utilization of SELENOP by peripheral tissues to synthesize selenium-containing antioxidant enzymes. Regarding the concept of exercise-induced hormesis, previous literature reported that the physiological amount of ROS produced during exercise can stimulate antioxidant enzyme expression in skeletal muscle[39-41] to restore intracellular redox homeo-stasis and protect from harmful oxidative damage. It was also reported that regular endurance exercise training increases GPX1 levels in skeletal muscles by 20%–177%.[40] Furthermore, a previous paper reported that serum selenium concentrations was lower in professional athletes to synthesize GPX enzymes in skeletal muscles.[23] As SELENOP is the major selenium supplier for synthesizing intracellular selenium-containing antioxidant enzymes, the cellular uptake and utilization of SELENOP may be augmented during antioxidant adaptation in chronic exercise, lowering its plasma level. In other words, our data suggest that SELENOP may have a particular crucial physiological role in the exercise-induced hormesis effect of habitual physical training. Further in-vivo and in-vitro uptake studies are recommended to address this hypothesis.

As expected, the leucocyte mtDNA CN was found to be significantly higher in the regularly-exercising than non-exercising individuals. That finding is consistent with the previous report[25] supporting the effect of physical exercise on mitochondrial biogenesis. Peroxisome-proliferator-activated receptor γ co-activator-1α (PGC-1α), the master regulator of mitochondrial biogenesis and exercise-induced H_2O_2 production in skeletal muscles, was reported to increase PGC-1α expression through AMPK activation.[42] Then PGC-1α binds to and co-activates the transcriptional function of nuclear respiratory factors 1 (NRF-1) on the promoter for mitochondrial transcription factor A (Tfam)[12] to induce mtDNA replication. Therefore, transient induction of oxidative stress during physical exercise activates mitochondrial biogenesis via the AMPK-PGC-1α signaling cascade to improve mitochondrial quantity with higher oxidative capacity and ATP production during the physiological state of increased metabolic demand.

Although it was not statistically significant, we found a trend of inverse correlation between antioxidant SELENOP levels and mtDNA CN in our population. The previous report also indicated higher mitochondrial DNA content in the skeletal muscle of trained SELENOP deficient mice than its wild-type counterparts.[16] Therefore, we assume that the relationship between SELENOP and mtDNA CN might be bi-directional depending on metabolic conditions. During physiological adaptation of regular exercise, increased mitochondrial numbers with accelerated oxygen consumption and subsequent ROS production by physical exercise may lower plasma SELENOP level due to increased utilization for compensated antioxidant enzyme synthesis. On the other hand, when SELENOP is over-expressed, the over-activity of SELENOP and its reductive stress may suppress exercise-induced mitochondrial biogenesis in skeletal muscle.[16] Failure to reach a statistically significant level in our study may be due to the fact that, contrary to the study of Misu et al,[16] the participants in our study were healthy volunteers with plasma SELENOP levels within

the physiological range. Therefore, little variations in plasma SELENOP levels among participants, as well as the small sample size, may result in failure to achieve a statistically significant correlation between the two variables. Large-scale clinical studies involving those with insulin resistance are recommended for future perspectives.

Limitations of the study

Our study had certain limitations. We could not examine the course of plasma SELENOP changes with exercise duration because of our cross-sectional study design. A longitudinal study is recommended to analyze the time-dependent changes in plasma SELENOP level and its physiological significance from acute to habitual exercise.

CONCLUSIONS

In conclusion, our study found lower plasma SELENOP levels and higher leucocyte mtDNA CN in the regular-exercise group than the non-exercise group, which remained significant after stratifying the subjects into two age groups. These findings suggest that regular exercise training is an effective measure for improving mitochondrial function while lowering circulating SELENOP levels in humans. Further longitudinal studies are recommended for a better understanding of the role of SELENOP in exercise metabolism which can provide a new therapeutic approach to enhance the benefits of physical exercise in clinical settings.

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Statement of Authorship
All authors certified fulfillment of ICMJE authorship criteria.

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