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Oxidative stress is associated with weight gain in recipients at 12-months following kidney transplantation

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Abstract

Objective—Weight gain after kidney transplantation (Tx) is considered a risk factor for poor outcomes. Increased oxidative stress is associated with not only chronic renal disease and Tx, but also obesity and cardiovascular disease. The aim of this pilot study was to test whether oxidative stress is related to weight gain at 12-months after kidney Tx and to obtain preliminary insight into potential mechanisms involved.

Design & Methods—Recipients (n=33) were classified into two groups; weight loss and weight gain, based on their weight changes at 12-months post-transplant. Total antioxidant capacity (TAOC) and lipid peroxidation (TBARS) were measured to evaluate oxidative stress from plasma at baseline and 12-months. A secondary data analysis was conducted to identify potential gene regulation.

Results—Seventeen recipients lost (-6.63 ± 5.52 kg), and sixteen recipients gained weight (8.94 ± 6.18 kg). TAOC was significantly decreased at 12-months compared to baseline for the total group, however, there was no significant difference between groups at either time point. TBARS was higher in weight gain group, at both time points, and it was significantly higher at 12-months ($p=0.012$). Gene expression profiling analysis showed that 7 transcripts annotated to reactive oxygen species related genes in adipose tissue were expressed significantly lower in weight gain group at baseline, which might be a negative feedback mechanism to reduce oxidative stress.

Conclusion—These results may indicate that elevated oxidative stress (TBARS) is associated with weight gain after kidney Tx and that incorporating early clinical prevention strategies known to decrease oxidative stress could be recommended.

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Keywords

kidney transplantation; oxidative stress; weight gain

1. Introduction

Epidemiological data from the past decade suggest that the global burden of patients with renal failure who receive kidney transplantation exceeds 1.4 million and this number is growing by about 8% a year [1]. Rapid advances have been made in decreasing acute rejection rates and improving short-term graft survival in kidney transplant recipients with most centers reporting survival rates of greater than 90% at 1 year [2, 3]. However, long-term success has been difficult to accomplish as evident by the marginal increase in patients and graft survival rates over the past 15 years from a half-life of 6.6 years in 1989 to an approximately 8.8 years in 2005 [4].

Kidney transplantation recipients are highly exposed to the risk of oxidative stress, which is an imbalance between the generation of reactive oxygen species and the antioxidants defense system. Reactive oxygen species is easily increased by end stage renal disease, which most recipients commonly suffered for a long time before transplant. Ischemia-reperfusion injury and long-term use of immunosuppressant drugs after transplantation also increase oxidative stress [5]. Moreover, oxidative stress is known to play an important role in the pathogenesis of cancer, systemic inflammation, and cardiovascular diseases, which are common comorbidities of transplantation recipients [5, 6].

Oxidative stress is also associated with obesity, which commonly occurs following kidney transplantation. Although the mechanism is unclear, numerous studies indicate that oxidative stress is elevated overall in obese individuals compared to non-obese in the general population [7, 8]. For kidney transplant recipients, weight gain and obesity are significant issues, which might complicate long-term graft success and contribute to the development of cardiovascular disease [9, 10]. It is reported that kidney transplant recipients experience a mean weight gain of 10–12 kg during the first year after transplantation [9, 11]. Studies conducted to reveal the cause of the dangerous weight gain have been inconclusive attributing it to a combination of multiple factors. Three primary factors, inappropriate dietary intake, reduced physical activity, and immunosuppressant drug therapy, were frequently considered as either its cause or therapeutic solution [12, 13].

Recently, we explored a potential genomic contribution by examining the association between gene expression from adipose tissue obtained during kidney transplantation and weight gain after transplantation [14]. This study reported that expression levels of 1,553 genes were significantly associated with weight change. In the gene list related to weight gain, there were oxidative stress-related genes including NADH dehydrogenase, ATP synthase, and glutathione s-transferase kappa 1. It was reported that these genes might affect to weight changes in the animal study and clinical trials [15–17].

Based on these findings, we hypothesized that oxidative stress is associated with weight gain after kidney transplantation. In order to test our hypothesis, we conducted an exploratory

study to measure and compare oxidative stress level from plasma obtained at the time of transplantation (baseline) and 12-months after transplantation in two groups, recipients who gained vs. those who lost weight in 12-months.

2. Materials and methods

From 2006 to 2011, investigators from a regional MidSouth transplant institute recruited 153 kidney transplant recipients to participate in a larger study examining genetic and environmental factors (e.g. diet and physical activity) associated with weight gain following transplantation. This study is a substudy with 37 recipients whose plasma samples were collected at both baseline and 12-months after transplantation. Four recipients were excluded due to their multi-racial ancestry, thus, final analysis was done with 33 kidney transplantation recipients.

All adults approved for kidney transplantation surgery at the transplant institute were eligible for participation in the parent study regardless of race and gender. However, recipients were excluded if at time of kidney transplantation they were taking prednisone or other immunosuppressant therapy, to control for any effect of pre-transplantation immunosuppressant therapy on baseline weight. This research was approved by the University of Tennessee Health Science Center Institutional Review Board and the Office of Human Subject Research Protection at the National Institution of Health. Written informed consent was obtained from all recipients.

Demographic features and clinical information including age, gender, race, weight, body mass index (BMI; kg/m^2), laboratory results and medication history were obtained from all participants at the time of transplant (baseline) and 12-months after transplant. Dietary data were obtained by experienced research team members who were trained in gathering recall data. Briefly, interviews were conducted approximately two weeks following transplantation because this period most likely reflected the return to regular eating patterns. Physical activity recall was elicited by telephone interview using the well-established Physical Activity Report [18]. Based on participants' response, five subsets of physical activity (sleep, light, moderate, hard, and very hard) were recorded, and average energy expenditure was determined with it.

Blood samples were collected from the antecubital vein into EDTA and sodium-heparin tubes at baseline and 12-months after transplantation. Plasma was separated immediately after the blood collection by centrifugation at 1,000g for 10min at 4°C and stored at -80°C until assay.

The ABTS assay was used to determine the total antioxidants capacity (TAOC). This assay relies on the ability of antioxidants in the plasma to inhibit the oxidation of 3-ethylbenzothiazole-6-sulfonate (ABTS) by metmyoglobin. Antioxidants suppressed the production of the radical cation in a concentration dependent manner and the color intensity decreases proportionally, which was determined at 405nm with SpectraMax® M3 Microplate Reader (Molecular Devices Corp., Sunnyvale, CA). Trolox, a water-soluble vitamin E analogue, serves as a standard or control antioxidant, and the capacity of the

antioxidants was quantified as mM Trolox equivalents. Detailed procedure was followed by manufacturer's direction (Sigma-Aldrich, St. Louis, MO).

The thiobarbituric acid reactive substances (TBARS) assay was conducted to measure the lipid peroxidation contents in plasma. It was determined by malondialdehyde (MDA) production and assayed based on the reaction with thiobarbituric acid (TBA) and MDA. The TBA-MDA adducts formed by the reaction under high temperature and acidic condition were measured fluorometrically at an excitation wavelength of 530nm and an emission wavelength of 550nm with SpectraMax® M3 Microplate Reader (Molecular Devices Corp., Sunnyvale, CA). Readings were converted into μM using the calibration curve. Detailed procedure was followed by manufacturer's direction (Cayman, Ann Arbor, MI).

To explore the gene expression profiles of oxidative stress-related genes from kidney transplantation recipients, data obtained from a previous substudy were analyzed. The study was fully described by Cashion et al. [14]. In the previous study, gene expression profiling was conducted with GeneChip Human Gene 1.0 ST Arrays (Affymetrix Inc, Santa Clara, CA) from adipose tissue and whole blood obtained at the time of transplant. Among twenty six recipients of the previous study, twelve (46.15%) were included in the current study. Briefly, the subject characteristics ($n=26$) are; eleven were men (42.13%), fifteen were African American (57.69%), and mean age was 47.73 ± 4.07 years old. Recipients gained a mean of 8.15 ± 21.01 kg by 12-months post-transplant; ten recipients showed weight loss (-10.34 ± 11.65 kg) and sixteen recipients showed weight gain (19.71 ± 16.85 kg, $p=0.000049$). Age, gender, race and immunosuppressant drugs were not significantly different between weight loss and weight gain groups. Oxidative stress-related genes were selected using DAVID bioinformatics resources (<http://david.abcc.ncifcrf.gov/home.jsp>) [19]. From 33,297 transcripts profile, 648 genes were chosen from oxidation reduction process category of Gene Ontology (632 genes) and oxidoreductase category of SP PIR KEYWORDS (557 genes). Then, 648 genes were classified with Kyoto Encyclopedia of Genes and Genomes (KEGG) into 8 categories, and 92 genes were selected from oxidative phosphorylation and glutathione metabolism category. The expression of those genes was compared between weight loss group and weight gain group.

Independent/paired t-test and Chi-square test were used to identify significant differences. Pearson product moment correlation was used to estimate the relationship between TBARS and body weight. Differences were considered to be statistically significant when $p < 0.05$. Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL).

3. Results

Thirty-three transplant recipients were included in this study and their general characteristics are given in Table 1; twenty-one were men (63.64%), fifteen were African American (45.45%) and mean age was 52.24 ± 10.42 years old. Body weight and BMI were measured at both baseline and 12-months. It was shown that 66.67% were overweight (BMI, 25.00 to 29.99 kg/m^2) or obese (BMI, 30.00 kg/m^2) at 12-months after transplantation.

Recipients were classified into two groups by the change of body weight in 12-months (Table 1 and Figure 1). Among 33 recipients, seventeen recipients showed weight loss

(-6.63 ± 5.52 kg), whereas sixteen recipients showed weight gain (8.94 ± 6.18 kg) at 12-months post-transplant. Most importantly, although all recipients were at a normal BMI, or slightly above, over 36% ($n=12$) gained greater than 5% of their baseline weight and 27% gained greater than 10% ($n=9$) of their baseline weight. This demonstrates the significant increase in weight experienced by some recipients, which can result in adverse comorbid conditions. BMI and body weight were not significantly different at baseline between groups, however as expected, at 12-months, both were significantly higher in weight gain group.

Other demographic characteristics including age, race, gender, hypertension, and diabetes status were not found to be significantly different between groups (Table 1). A standard immunosuppressant post-transplant protocol was followed, therefore, the medications were not significantly different between weight loss and weight gain groups. In general, prednisone (20 mg/day) was prescribed at transplant and tapered to 5 mg/day by 12-months. Over 90% of recipients were also taking tacrolimus and mycophenolate at either time point. No recipients were prescribed cyclosporine.

Energy intake did not significantly change from baseline to 12-months for the total group. Interestingly, it was significantly higher in weight gain group at baseline compared to the weight loss group ($p=0.039$), however, this difference disappeared at 12-months. The average daily energy expenditure significantly increased from baseline to 12-months for the total group ($p=0.018$). When examined by groups, it was significantly higher in weight loss group at baseline compared to weight gain group ($p=0.009$), however, there was no difference between groups at 12-months.

To determine whether oxidative stress was associated with body weight, total antioxidant capacity (TAOC) and lipid peroxidation (TBARS) were measured from plasma which was collected at baseline and 12-months after transplantation (Figure 2). Overall, TAOC was significantly decreased at 12-months (1.79 ± 0.98 mM) compared to baseline (2.26 ± 1.37 mM) for the total group ($p=0.018$), however, it was not significantly different between weight loss and gain groups at both baseline and 12-months. TBARS did not significantly change from baseline to 12 months for the total group (1.09 ± 0.79 μ M at baseline and 1.13 ± 0.68 μ M at 12-months, $p=0.787$). However, when examined by groups, it was relatively higher in the weight gain group compared to the weight loss group at baseline (1.32 ± 0.79 μ M and 0.85 ± 0.72 μ M, respectively; $p=0.082$) and significantly higher in the weight gain group compared to the weight loss group at 12-months (1.41 ± 0.18 μ M and 0.84 ± 0.12 μ M, respectively; $p=0.012$).

Baseline body weight was not correlated with baseline TBARS ($r=0.053$, $p=0.769$), however, 12-months body weight was significantly correlated with 12-months TBARS ($r=0.453$, $p=0.008$). Correlation between immunosuppressant and TBARS or TAOC was also tested. Dosage of each drug was not correlated with TBARS or TAOC at both time points, although change of tacrolimus dose was significantly correlated with change of TBARS ($r=-0.398$, $p=0.022$).

To begin exploring mechanistic pathways, we conducted a secondary database analysis from our previous data [14] to identify oxidative stress-related genes differentially expressed between transplant recipients who lost and gained weight from two different tissues, adipose tissue and whole blood (Table 2). In the adipose tissue gene expression profile, transcripts annotated to seven genes (*MT-TK*, *NDUFS2*, *SDHD*, *UQCRC2*, *NDUFA5*, *ND2*, and *CCDC104*) and one unannotated transcript were expressed significantly lower in weight gain group. Interestingly, transcripts annotated to three genes (*IDH1*, *COX1* and *CYTB*) showed opposite trend in the whole blood gene expression profile, without statistical significance, i.e. higher expression in weight gain group.

4. Discussion

Significant weight gain was a post-transplant reality for over 30% of our sample, with approximately 27% gaining more than 10% of their baseline weight. For those individuals, complications associated with oxidative stress are concern. In this study, we demonstrated that oxidative stress is associated with weight change at 12-months after kidney transplantation. We compared oxidative stress levels by measuring total antioxidant capacity (TAOC) and lipid peroxidation (TBARS) between weight loss and weight gain groups at baseline and 12-months of post-transplant. TAOC was not different between groups at either time point, however, TBARS showed a noticeable higher trend in the weight gain group at baseline, and was significantly greater at 12-months, compared to the weight loss group. Weight gain at 12-months was not related to immunosuppressant administration, energy intake and physical activity between groups. This study would be the first demonstration to show that oxidative stress (TBARS) is associated with body weight at 12-months after kidney transplantation.

Our findings are congruent with other studies which reported decreased TAOC at 12 days [20], 6 months [21] and 12 months [22] after kidney transplantation. However, the mechanism which induced decreased TAOC is controversial. It was suggested that it might be caused by the reduction of uric acid which may lead to major variations of the internal redox state [20]. Meanwhile, it was also suggested that TAOC may not be related or insufficiently sensitive to oxidative stress after kidney transplantation [21, 22]. Immunosuppressant is considered to be an important factor for the change of TAOC, however, the effect of immunosuppressant is also controversial. It was reported that recipients treated with cyclosporine had significantly greater TAOC compared with those treated with tacrolimus [23], while another study found no significant difference in TAOC between recipients treated with cyclosporine versus tacrolimus [22]. Because over 90% of recipients in our study were treated with tacrolimus and mycophenolate, we could not define the role of each medication to TAOC in this study. Since previous study results lack consensus on whether TAOC is a useful parameter for oxidative stress and how immunosuppressant therapies affect TAOC, other parameters such as TBARS need to be considered along with TAOC to determine the role of oxidative stress on weight gain.

The TBARS was not significantly changed at 12-months compared to baseline for all recipients. In contrast, other studies reported significantly increased TBARS at 2 weeks [24] and 52 weeks [25] or significantly decreased TBARS at 28 days [26] and 1 year [27] after

kidney transplantation. The lack of consistency in results might be explained by the variation among studies in measurement time point and characteristics of the population, though most studies overall suggested that TBARS change might be related to the type of immunosuppressant administration. It is known that calcineurin-inhibitors, such as cyclosporine and tacrolimus may induce oxidative stress, in contrast, mycophenolate ameliorates it [5], although tacrolimus is known to have more beneficial effects on the level of oxidative stress compared to cyclosporine [28]. In our study, we observed that immunosuppressant therapies were not correlated to TBARS at both time points, however a decrease in tacrolimus dosage was associated with an increase in TBARS, which is consistent with finding with another study [28]. Considering all recipients were treated with mycophenolate and tacrolimus, it may not represent the direct correlation between tacrolimus and TBARS. Taken together with decreased TAOC, it may suggest that oxidative stress might be increased at 12-months post-transplant in this study for the total group, although the background mechanism related to TBARS is not clear.

Oxidative stress is an important factor related to weight gain or obesity, which is supported by the results of animal and clinical studies [7, 29]. Many studies in the general population have shown that TBARS was significantly higher in obese groups, and it is known that accumulation of oxidative stress might be induced by increased adipose tissue at the early stage of weight gain [7, 30]. For kidney transplant recipients, Chan et al. proposed that increased BMI induces sarcopenic obesity, then decreased muscle mass may cause impaired muscle-based oxidative mechanism, which leads to break down oxidative defense system [31]. Our study also showed that TBARS was significantly higher in the weight gain group at 12-months as compared to the weight loss group, but that same significant difference was not present at baseline. Notably there is a similar pattern seen with body weight and BMI, such that there is a significant difference between groups at 12-months that was not present at baseline. Based on this finding, we suggest that TBARS is associated with weight gain after kidney transplantation.

Many studies have been conducted with transplant recipients to determine factors related to weight gain. Some studies demonstrated that gender differences may impact weight gain or obesity [32, 33]. And some studies showed African Americans gain weight easier than Caucasians [34, 35]. In our study, gender and race were not statistically different between weight loss and gain group suggesting that it may not be a primary factor affecting weight changes in the first year following kidney transplantation. Energy intake is also considered as a key factor to induce weight gain and obesity as the improved appetite and sense of well-being may lead to augmented caloric intake. There are some reports that nutrition intervention may assist to inhibit weight gain after transplantation [36, 37]. In our study, there was no difference in energy intake between weight loss and weight gain group. Rather, energy intake was slightly decreased in weight gain group at 12-months. This could be explained by the possibility that some recipients may make an effort to reduce their body weight to avoid complications from obesity, which could be supported by the significantly increased average daily energy expenditure in the weight gain group at 12 month ($p=0.002$). Therefore, increased energy intake and decreased physical activity could be ruled out as a factor for the weight gain in our population. Immunosuppressant therapy is also a candidate to increase the body weight after transplantation, however, its effect on weight gain is also

controversial [13, 38, 39]. In our study, the application dosage of prednisone, tacrolimus and mycophenolate was statistically similar between groups. Taken together, we suggest that gender, race, energy intake, physical activity and immunosuppressant medication may not have any significant impact on weight changes in this study.

Expression of reactive oxygen species (ROS) related gene profile was different between adipose tissue and whole blood. Unexpectedly, the expression of ROS related genes in the adipose tissue was significantly lowered in weight gain group. On the contrary, in the whole blood, the expression of ROS related genes showed increased tendency in weight gain group. Similar trends were shown in both oxidative stress (TBARS) and body weight, which were higher in weight gain group at the baseline. Based on this, we suggest that increased body weight might be associated with increased oxidative stress at the baseline. And these modestly activated factors in whole blood and body weight might induce a lower expression of ROS related genes in adipose tissue in weight gain group. We suggest that it might be because of a negative feedback mechanism that maintains the redox homeostasis after transplantation. Due to this mechanism in our body, excessive increase of oxidative stress after transplantation could be avoided in the weight gain group. It may support our result that TBARS was almost the same at 12-months in the weight gain group.

In this study, our small sample size is a limitation. Another limitation is the use of different samples to obtain gene expression data, even though almost half of recipients are in both studies. A larger population is needed to investigate how oxidative stress is associated with weight gain after transplantation.

Our study is the first to show that oxidative stress is increased in kidney transplant recipients who gain weight. We suggest that oxidative stress may play a role in weight gain after kidney transplantation. In combination, weight gain and oxidative stress, further increases the risk of cardiovascular complications and may explain some of the underlying physiology related to these events. It may suggest the future therapeutic direction, such as antioxidant therapy to prevent cardiovascular complications after kidney transplantation.

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Highlights

- Recipients who gained vs. lost weight after kidney transplant were compared.
- Total antioxidant capacity and lipid peroxidation were measured from plasma.
- Lipid peroxidation (TBARS) was significantly increased in weight gain group.
- Expression of ROS-related genes were explored.
- Elevated oxidative stress might be associated with post-transplant weight gain.

Figure 1. Changes of weight and body mass index (BMI) at 12-months after kidney transplantation

Body weight (A) and BMI (B) were significantly increased in weight gain group compared to weight loss group after 12-months of kidney transplantation. Values are means±SE.

Figure 2. Changes of total antioxidant capacity (TAOC) and lipid peroxidation (TBARS) at 12-months after kidney transplantation

A. Total antioxidant capacity was significantly decreased at 12-months compared to baseline from all recipients, however, it was not significantly different between weight loss and weight gain groups at baseline and 12-months after transplantation. B. TBARS was increased in weight gain group in baseline of transplantation ($p=0.082$) and it was significantly increased at 12-months after transplantation ($p=0.012$). Values are means $\pm$ SE.

Table 1

Characteristics of recipients at time of transplant (baseline) and 12-months after transplant

Characteristics		Entire recipients n=33		Weight loss group n=16		Weight gain group n=17	
		baseline	12-months	baseline	12-months	baseline	12-months
Age (year)		52.24±10.42		55.88±9.47		48.82±10.36	
Gender (Male)	n (%)	21 (63.64%)		11 (68.75%)		10 (58.82%)	
Race	AA, n (%)	15 (45.45%)		8 (50.00%)		7 (41.18%)	
	C, n (%)	18 (54.55%)		8 (50.00%)		10 (58.82%)	
Hypertension	n (%)	33 (100.00%)	29 (93.55%)	16 (100.00%)	14 (93.33%)	17 (100.00%)	15 (93.75%)
Diabetes	n (%)	5 (15.15%)	8 (25.00%)	2 (12.50%)	5 (31.25%)	3 (17.65%)	3 (17.65%)
Body weight (Kg)		83.57±18.42	84.96±23.04	78.86±18.55 [#]	72.24±16.99 ^{#*}	88.00±17.69 [#]	96.94±21.84 ^{#*}
Body weight (Kg)		1.39±9.79		-6.63±5.52 [*]		8.94±6.18 [*]	
BMI (kg/m ²)		28.54±4.48	28.94±6.05	27.34±4.35 [#]	25.05±4.14 ^{#*}	29.67±4.43 [#]	32.60±5.26 ^{#*}
BMI (kg/m ²)		0.40±3.23		-2.30±1.90 [*]		2.94±1.83 [*]	
Prednisone (mg/day)		18.03±3.94 [#]	5.16±2.95 [#]	17.81±3.64 [#]	5.67±4.17 [#]	18.24±4.31 [#]	4.69±0.85 [#]
Tacrolimus (mg/day)		4.57±2.44	5.58±3.57	4.63±2.19	5.19±3.99	4.41±2.72	5.94±3.20
Mycophenolate (mg/day)		1769.70±343.37 [#]	1109.09±815.72 [#]	1770.00±406.74 [#]	953.75±616.13 [#]	1769.41±284.09 [#]	1255.29±963.37 [#]
Energy intake (cal)		1768.39±734.90	1791.16±772.91	1499.22±491.98 [*]	1578.46±558.34	2021.73±844.15 [*]	1991.36±902.60
Energy intake (cal)		22.78±964.54		79.24±652.12		-30.37±1206.56	
Ave. daily energy expenditure		32.34±1.30 [#]	34.25±4.32 [#]	32.93±1.33 [*]	35.35±5.85	31.79±1.01 ^{#*}	33.22±1.71 [#]
Ave. daily energy expenditure		1.91±4.41		2.42±6.18		1.44±1.65	

Note : Data are presented as mean±SD. SD=standard deviation, AA=African American, C=Caucasian, BMI=body mass index, kg=kilogram, m=meter, =change.

* means p<0.05 between weight loss and weight gain group.

means p<0.05 between baseline and 12-months in the same group.

Table 2
Candidate genes and their transcript expression level at baseline from adipose tissue and whole blood

from a subgroup of 26 recipients from the previous study [14], 46.15% were included in the current study.

	Gene Symbol	Gene Title	Weight loss group	Weight gain group	p value
Adipose tissue	NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)	9.58±0.22	9.33±0.20	0.00834712
	UQCRC2	Ubiquinol-cytochrome c reductase core protein II	10.67±0.21	10.42±0.31	0.02713103
	MT-TK	Mitochondrially encoded tRNA lysine	10.44±0.46	9.98±0.51	0.02814656
	SDHD	Succinate dehydrogenase complex, subunit D, integral membrane protein	8.49±0.24	8.23±0.32	0.03643888
	MTND2	Mitochondrially encoded NADH dehydrogenase 2	11.43±0.38	11.12±0.34	0.04000202
	NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	8.56±0.15	8.33±0.32	0.04238961
	CCDC104	Coiled-coil domain containing 104	12.81±0.20	12.64±0.19	0.04867911
	IDH1	Isocitrate dehydrogenase 1 (NADP+), soluble	9.33±0.46	9.61±0.25	0.05656491
Whole blood	COX1	Cytochrome c oxidase subunit 1	13.52±0.16	13.62±0.10	0.08233270
	CYTB	Cytochrome b	13.52±0.17	13.62±0.12	0.09276942