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Effects of Age and Tocotrienol-Rich Fraction on Mitochondrial Respiratory Complexes in the Hippocampus of Rats

(Kesan Umur dan Fraksi Kaya Tokotrienol pada Kompleks Respirasi Mitokondria dalam Hipokampus Tikus)

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ABSTRACT

Mitochondrial dysfunction is common in the brain with age. Prevention of mitochondrial dysfunction at an early age may protect the brain against neurodegeneration in later life. Tocotrienol-rich fraction (TRF) has been reported to be neuroprotective in old rats, but its effect remains unknown for middle-aged animals. This study aimed to determine the effect of TRF on activities of mitochondrial respiratory chain complexes in the hippocampus of middle-aged rats. Male Sprague Dawley rats were divided into 4 groups: young control (3 months old), adult control (12 months old), adult rats supplemented with palm kernel oil (PKO) as the vehicle, and adult rats supplemented with TRF by gavage at 200 mg/kg body weight/day for 3 months. At the end of the supplementation, activities of complex I, I+III, II, II+III, III, IV, and citrate synthase in the isolated mitochondria of the hippocampus were measured by spectrophotometry. Complex II activity was higher, while citrate synthase activity was lower in adult rats than in young rats. A decrease of citrate synthase activity suggests loss of mitochondrial mass and intactness in the hippocampus at middle age. Interestingly, PKO-treated adult rats had lower complex I and IV activities, but higher complex I+III activity than adult control rats. These findings indicate PKO modulated activities of the complexes. In TRF-treated adult rats, the complex I activity was higher, while the complex IV activity was lower than PKO-treated adult rats. TRF restored the complex I activity and may have the potential to reverse complex I deficiency.

Keywords: Aging; brain; mitochondria; respiratory complex; tocotrienols

ABSTRAK

Mitokondria disfungsi dalam otak biasanya berlaku pada usia tua. Halangan terhadap disfungsi tersebut pada waktu muda mungkin berupaya melindungi otak daripada neurodegenerasi semasa berusia. Fraksi kaya tokotrienol (TRF) dilaporkan bersifat pelindung neuron pada tikus tua, namun kesan tersebut tidak diketahui pada haiwan yang berumur pertengahan. Kajian ini bertujuan untuk menentukan kesan TRF terhadap aktiviti kompleks rantaian respiratori mitokondria dalam hipokampus tikus berumur pertengahan. Tikus jantan Sprague Dawley dibahagikan kepada 4 kumpulan: kawalan muda (berusia 3 bulan), kawalan dewasa (berusia 12 bulan), tikus dewasa dengan suplementasi minyak isirung sawit (PKO) sebagai pembawa dan tikus dewasa dengan suplementasi TRF (200 mg/kg) melalui gavaj untuk 3 bulan. Pada hujung suplementasi, aktiviti kompleks I, I+III, II, II+III, III, IV dan sitrat sintase dalam mitokondria yang diasingkan daripada hipokampus diukur dengan spektrofotometri. Aktiviti kompleks II adalah lebih tinggi, manakala sitrat sintase adalah lebih rendah pada tikus dewasa berbanding dengan yang muda. Penurunan aktiviti sitrat sintase mencadangkan kehilangan bilangan dan keutuhan mitokondria dalam hipokampus pada umur pertengahan. Kumpulan PKO mempunyai aktiviti kompleks I dan IV yang lebih rendah, manakala aktiviti kompleks I+III yang lebih tinggi berbanding dengan kawalan tikus dewasa. Hasil ini menunjukkan bahawa PKO mengawal atur aktiviti kompleks tersebut. Dalam kumpulan TRF, aktiviti kompleks I adalah lebih tinggi, manakala aktiviti kompleks IV adalah lebih rendah berbanding dengan kumpulan PKO. TRF telah memulihkan aktiviti kompleks I dan berpotensi menghalang keadaan yang disebabkan oleh defisiensi kompleks I.

Kata kunci: Kompleks respirasi; mitokondria; otak; penuaan; tokotrienol

Introduction

Age is the major risk factor for neurodegenerative disorders such as Alzheimer's disease. Aging is defined as a progressive deterioration of physical, physiological, and psychological fitness due to the accumulation of molecular damages overtimes. Mitochondrial dysfunction

has been regarded as one of the hallmarks of aging (López-Otín et al. 2013). The mitochondrial free radical theory of aging proposes that mitochondrial dysfunction leads to the overproduction of reactive oxygen species (ROS), which triggers further cellular damage (Grimm & Eckert 2017; Sun et al. 2016). Therefore, the study of mitochondrial impairments has been the center of interest in understanding the basis of healthy aging and age-associated disorders. The main role of mitochondria is to provide energy to the cell through oxidative phosphorylation (OXPHOS), while ROS are by-products of the process (Abdul Razak et al. 2018). OXPHOS is the process of ATP formation due to electron transfer from NADH or $FADH_2$ to O_2 by a series of electron carriers. The electron transport chain (ETC) is comprised of a series of large protein complexes, namely complex I (NADH: ubiquinone oxidoreductase), II (succinate dehydrogenase), III (coenzyme Q: cytochrome c), and IV (cytochrome c oxidase), which span across the mitochondrial inner membrane. Complexes I and II accept electrons from NADH and $FADH_2$, respectively, and transfer the electrons to complex III through ubiquinone. Then, complex III transfers the electrons to complex IV via cytochrome c, and eventually, the electrons are transferred to O_2 to form water. Protons are pumped into intermembrane space by complexes I, III, and IV during the electron transfer process. The generated proton gradient drives the synthesis of ATP (Abdul Razak et al. 2018).

The brain is particularly susceptible to mitochondrial impairments as it is a high-energy-demanding organ that utilizes approximately 20% of the body's basal oxygen (Grimm & Eckert 2017). Disturbances in mitochondrial bioenergetics are widely reported in the brain with increasing age (Hagl et al. 2013; Navarro et al. 2002, 2008; Sandhu & Kaur 2003). The respiratory chain's efficiency diminishes with age, which leads to the reduction of ATP generation and increment of electron leakage. However, changes in mitochondrial bioenergetics in different brain regions during middle adulthood are not well studied. Understanding such changes may provide new insights in preventing the development of neurodegeneration in later life due to prolong mitochondrial dysfunction. Various ways have been proposed to maintain healthy aging. Interventions such as endurance training (Gusdon et al. 2017; Menshikova et al. 2006) and caloric restriction (Amigo et al. 2017; Lanza et al. 2012) have been shown to mitigate the deterioration effect of aging. The involvement of ROS in mitochondrial dysfunction suggests the possibility of using antioxidants as prevention measures. Dietary antioxidant supplements may have a protective effect against age-associated mitochondrial impairments. Vitamin E is a lipid-soluble antioxidant that consists of 8 members (tocopherols and tocotrienols of alpha, beta, gamma, and delta forms). Each member may exhibit distinct biological effects that other members may not share, while tocotrienols have been reported to be more potent than tocopherols (Tan et al. 2016). Tocotrienol-rich fraction (TRF) is a rich source of tocotrienols which contains mainly alpha-tocotrienol, alpha-tocopherol, and gamma-tocotrienol. Previous studies have shown that TRF improved cognitive function in Alzheimer disease mouse model (Durani et al. 2018; Ibrahim et al. 2017) and aged rats (24 months old) (Taridi et al. 2014). Prevention of neurodegeneration by TRF at an early age may serve as a strategy for managing the ever-increasing aging population. However, the effect of TRF on mitochondrial bioenergetics in the brain remains unclear regardless of age group. Therefore, this study aimed to investigate the effect of TRF on activities of mitochondrial respiratory chain complexes in the hippocampus of middle-aged rats.

Materials and Methods

Animals and Treatment

Male Sprague Dawley rats were purchased from the Animal Unit (LARU), Universiti Kebangsaan Malaysia (UKM) at 3 and 12 months old. TRF and vitamin E-stripped palm kernel oil (PKO) were supplied by Sime Darby Research Sdn Bhd (Kuala Lumpur, Malaysia). TRF used in this study consisted of 30.5% d-alpha-tocotrienol, 30.2% d-alpha-tocotrienol, 27.9% d-alpha-tocopherol, 8.6% d-delta-tocotrienol, and 2.9% d-beta-tocotrienol. PKO was used as vehicle for TRF, and its fatty acid composition was made up of 42.1% C12, 21.8% C18-1, 12.7% C14, 9.2% C16, 4% C8, and 3.4% C10. The rats were divided into 4 groups of 3 rats per group: 3-month-old control rats (young rats), 12-month-old control rats (adult rats), vehicle-supplemented 12-month-old rats (PKO-treated adult rats), and TRF-supplemented 12-month-old rats (TRF-treated adult rats). TRF was supplemented to the rats by oral gavage at 200 mg/kg body weight/day for 3 months according to the previous study (Taridi et al. 2014). Control rats were supplemented with water. The rats were housed in individually ventilated cages at 22 °C under 12:12 h light/dark cycle with access to food and water *ad libitum*. The animal works were approved by the Animal Ethical Committee of UKM with the approval code: BIOK/PP/2018/JEN KIT/26-SEPT./951-SEPT.-2018-JULY-2019-AR-CAT2.

ISOLATION OF MITOCHONDRIA

At the end of the supplementation, the rats were sacrificed, brains were dissected out, hippocampi were removed from the brain and stored at -80 °C until use. Isolation of mitochondria was performed as described previously (Spinazzi et al. 2012). Briefly, the hippocampus was cut into smaller pieces and transferred into a Dounce homogenizer tube with 1 mL cold homogenization buffer (250 mM sucrose, 20 mM Tris, 40 mM KCl, 2 mM EGTA, pH 7.4) for every 100 mg wet weight. The tissue was homogenized with a loose pestle for 10 cycles followed by a tight pestle for 10 cycles. The homogenate was centrifuged at 600 × g for 10 min at 4 °C. The pellet represented the mitochondrial-enriched fraction was resuspended with 100 uL hypotonic Tris buffer (10 mM, pH 7.6) and stored at -80 °C until use. The protein concentration of the mitochondrial-enriched fraction was measured by BCA (Pierce, Thermo Scientific, Waltham MA, USA). The fraction was subjected to 3 cycles of freeze-thawing before used for measuring enzymatic activities.

MEASUREMENT OF ENZYMATIC ACTIVITY

Enzymatic activities of the mitochondrial complexes and citrate synthase were measured by spectrophotometry according to previous studies (Abdul Razak et al. 2019; Spinazzi et al. 2012). Reaction mixture for Complex I was consisted of 50 mM potassium phosphate (pH 7.5), 3 mg/mL essential fatty acid-free bovine serum albumin (BSA), 0.3 mM potassium cyanide, 0.1 mM NADH, 10 µM rotenone, 6 µM ubiquinone, and 2 µg mitochondrial isolate. The assay was read at 340 nm, 25 °C for 30 min in a kinetic mode with 30 s interval. The same reaction mixture without rotenone was prepared and regarded as total complex I activity.

Reaction mixture for Complex I+III was consisted of 50 mM potassium phosphate (pH 7.5), 1 mg/mL BSA, 0.3 mM potassium cyanide, 50 µM oxidized cytochrome c, 10 µM rotenone, 0.2 mM NADH, and 4 µg mitochondrial isolate. The assay was read at 550 nm, 25 °C for 30 min in a kinetic mode with 30 s interval. The same reaction mixture without rotenone was prepared and regarded as total complex I+III activity.

Reaction mixture for Complex II was consisted of 25 mM potassium phosphate (pH 7.5), 1 mg/mL BSA, 0.3 mM potassium cyanide, 20 mM succinate, 10 mM malonate, 0.002% DCPIP, 0.05 mM decylubiquinone, and 2 µg mitochondrial isolate. The assay was read at 600 nm, 25 °C for 30 min in a kinetic mode with 30 s interval. The same reaction mixture without malonate was prepared and regarded as total complex II activity.

The reaction mixture for Complex II+III consisted of 20 mM potassium phosphate (pH 7.5), 0.3 mM potassium cyanide, 10 mM succinate, 10 mM malonate, 50 µM oxidized cytochrome, and 4 µg mitochondrial isolate. The assay was read at 550 nm, 25 °C for 30 min in a kinetic mode with 30 s interval. The same reaction mixture without malonate was prepared and regarded as total complex II+III activity.

Reaction mixture for Complex III was consisted of 25 mM potassium phosphate (pH 7.5), 75 µM oxidized cytochrome c, 0.5 mM potassium cyanide, 0.1 mM EDTA pH 7.5, 0.025% Tween-20, 10 µg/mL antimycin A, 0.1 mM decylubiquinol and 0.5 µg mitochondrial isolate. The assay was read at 550 nm, 25 °C for 30 min in a kinetic mode with 30 s interval. The same reaction mixture without antimycin A was prepared and regarded as total complex III activity.

The reaction mixture for Complex IV consisted of 5 mM potassium phosphate (pH 7.0), 75 µM reduced cytochrome c, 0.3 mM potassium cyanide, and 0.5 µg mitochondrial isolate. The assay was read at 550 nm, 25 °C for 30 min in a kinetic mode with 30 s interval. The same reaction mixture without potassium cyanide was prepared and regarded as total complex IV activity.

The activity of the enzyme was obtained from the slope of the kinetic curve. The specific enzymatic activity for each complex was obtained by subtracting the reaction mixture with inhibitor (inhibitor-resistant activity) from the reaction mixture without inhibitor (total activity). For example in (1):

$$\text{Complex I-specific activity} = \text{slope } OD_{340} \text{ (without rotenone)} - \text{slope } OD_{340} \text{ (with rotenone)} \quad (1)$$

Reaction mixture for citrate synthase was consisted of 0.1 M Tris pH 8.0, 0.25% Triton X-100, 0.1 mM DTNB, 0.3 mM acetyl-CoA, 0.5 mM oxaloacetate, and 1 µg mitochondrial isolate. The assay was read at 412 nm, 25 °C for 30 min in a kinetic mode with 30 s interval.

The overall enzymatic activities had been normalized by the amount of protein input. To evaluate the complex's activity in a single mitochondrial unit, the overall complex activity was further normalized by citrate synthase activity.

STATISTICAL ANALYSIS

The enzymatic assays consisted of 3 biological replicates for each group. Each biological sample was further divided into 3 technical replicates. The difference between groups was evaluated by t-test. A p-value of <0.05 was considered statistically significant.

Results

Activity of citrate synthase

Citrate synthase activity was lower in adult rats than in young rats as show in Figure 1. There was no difference in the citrate synthase activity between TRF- and PKO-treated adult rats.

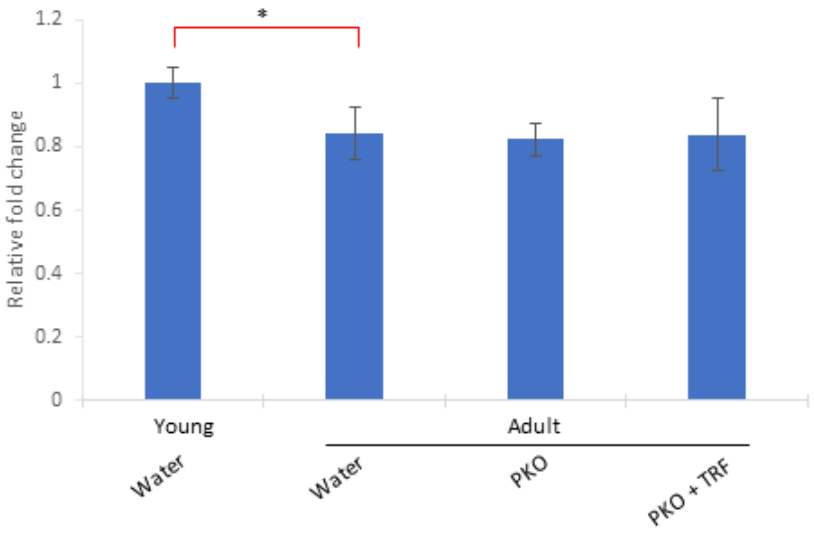

FIGURE 1. Citrate synthase activity in rat hippocampus. Young rats were supplemented with water, while adult rats were supplemented with water, PKO, or PKO+TRF. N = 3 biological replicates with 3 technical replicates each + SD; *p<0.05

Activities of complex I and I+III

Overall and normalized activities of complex I and complex I+III were no difference between young and adult rats as shown in Figure 2. The overall activity of complex I was lower in PKO-treated adult rats than in control or TRF-treated adult rats as shown in Figure 2(a). After normalization with citrate synthase activity, complex I activity in PKO-treated adult rats remained lower than adult control rats as shown in Figure 2(b). Before and after normalization with citrate synthase activity, complex I+III activity remained higher in the PKO-treated adult rats than adult control rats as shown in Figure 2(c) - 2(d).

Activities of complex II and II+III

The overall activity of complex II was higher in adult control rats than both young control and PKO-treated adult rats as shown in Figure 3(a). The result remained the same after normalization with citrate synthase activity as shown in Figure 3(b). There was no difference in complex II+III activity for all groups before and after normalization with citrate synthase activity as shown in Figure 3(c) - 3(d).

Activities of complex III and IV

Complex III activity remained unchanged for all groups before and after normalization with citrate synthase activity as shown in Figure 4(a) - 4(b). The overall activity of complex IV was lower in PKO-treated adult rats than adult control rats as shown in Figure 4(c). In comparison, TRF-treated adult rats had a lower overall complex IV activity than PKO-treated adult rats. No difference in complex IV was observed for all groups after normalization with citrate synthase activity as shown in Figure 4(d).

Discussion

Previously, the activities of complex I and II in the brain were evaluated by measuring the cactivities of complex I+III and II+III (Navarro et al. 2011, 2008, 2005)compared with young (4 mo. In this study, the individual activity of complex I and II, and the coupled

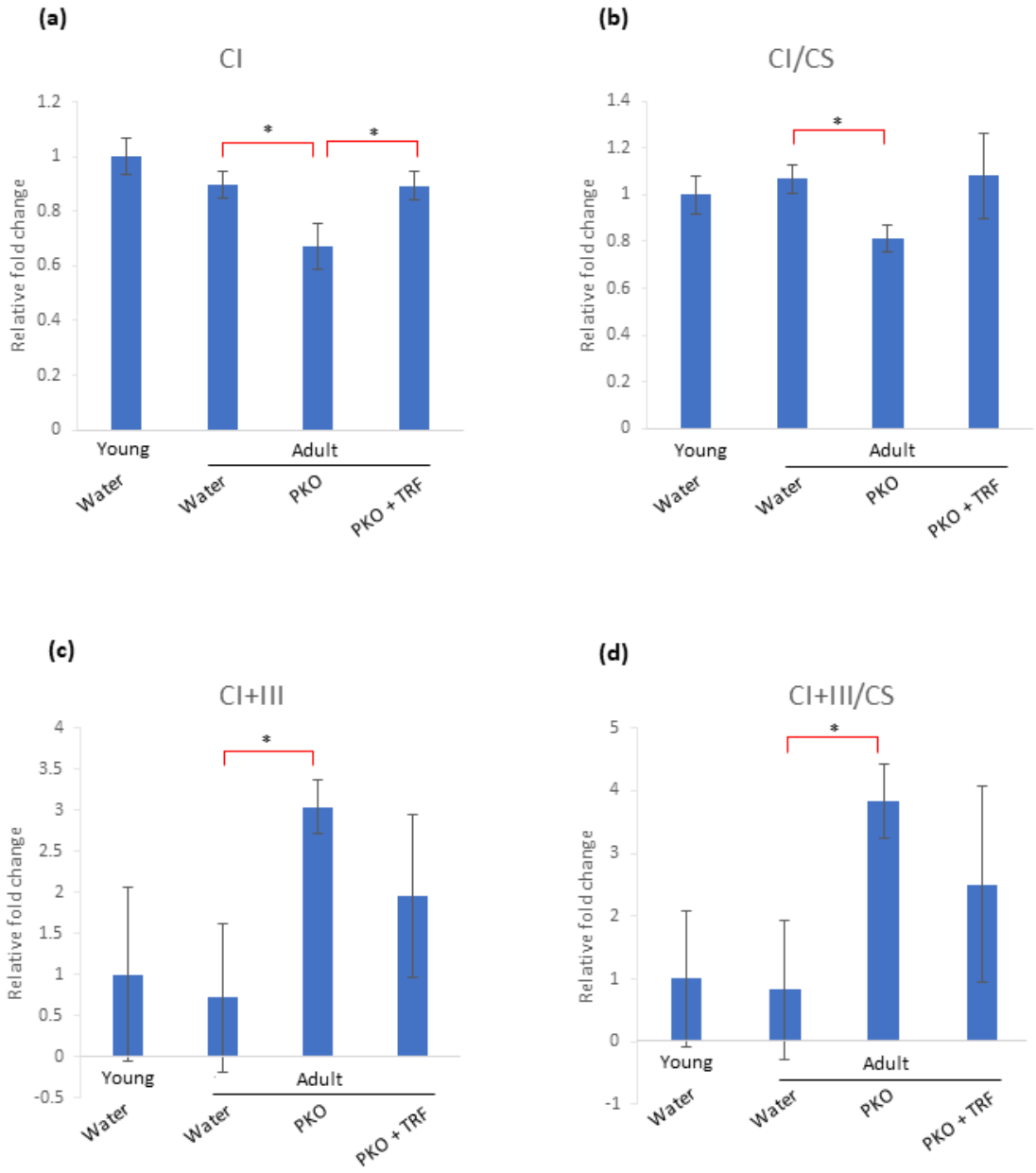

FIGURE 2. Complex I and I+III activities: (a) The overall activity of complex I, (b) activity of complex I normalized with citrate synthase activity, (c) overall activity of complex I+III, and (d) activity of complex I+III normalized with citrate synthase activity in rat hippocampus. Young rats were supplemented with water, while adult rats were supplemented with water, PKO, or PKO+TRF. N = 3 biological replicates with 3 technical replicates each + SD; *p<0.05

activity of complex I+III and II+III were evaluated to provide a more detailed description of the changes of mitochondrial respiratory chain complexes' activities with age. Activities of mitochondrial respiratory complexes were normalized to total proteins to represent the overall activity of the complex, while normalization to citrate synthase activity was to evaluate the complex activity in each mitochondrion.

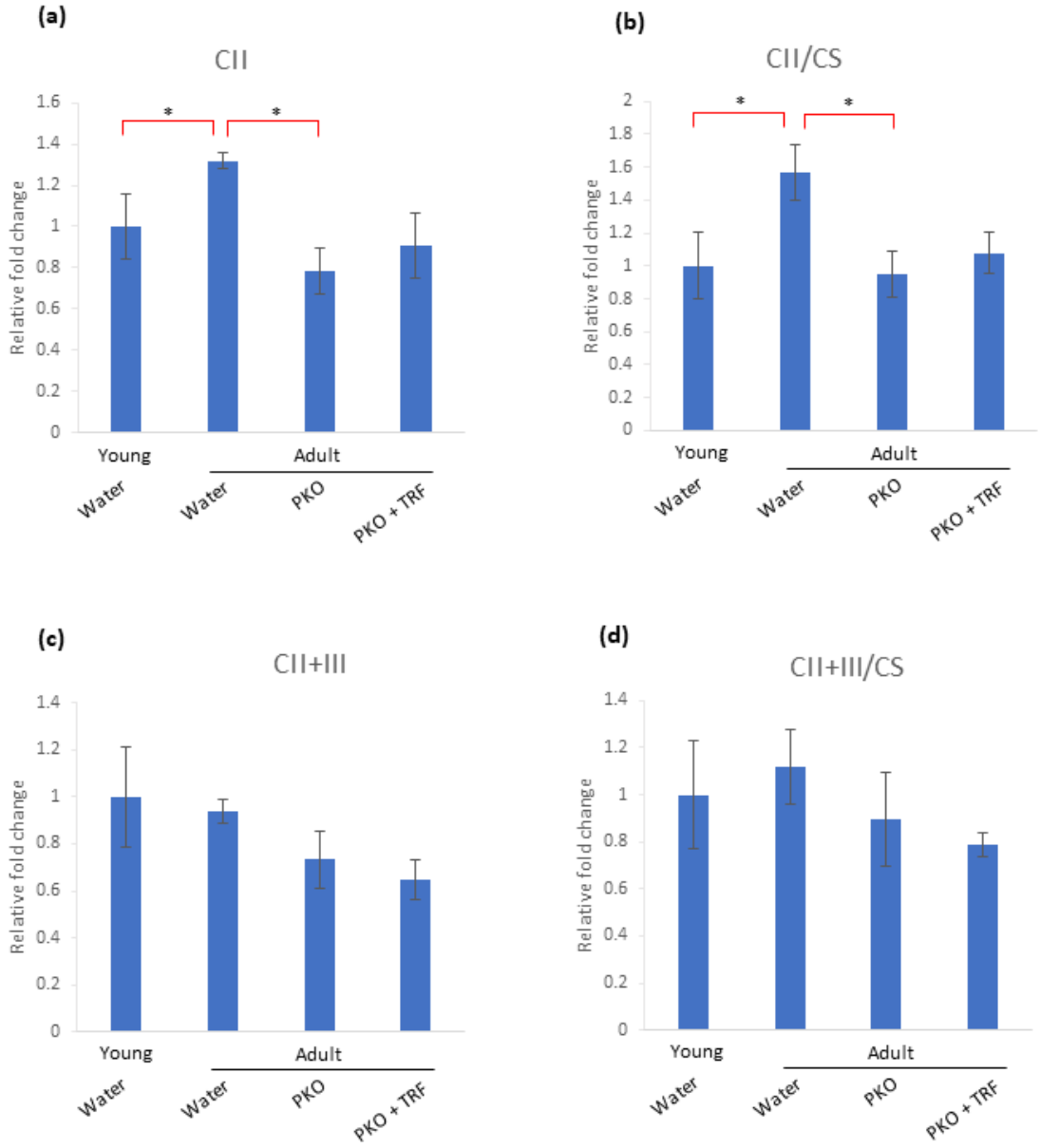

FIGURE 3. Complex II and II+III activities: (a) Overall activity of complex II, (b) activity of complex II normalized with citrate synthase activity, (c) overall activity of complex II+III, and (d) activity of complex II+III normalized with citrate synthase activity in rat hippocampus. Young rats were supplemented with water, while adult rats were supplemented with water, PKO or PKO+TRF. N = 3 biological replicates with 3 technical replicates each + SD; *p<0.05

Citrate synthase is localized in the matrix and not part of the ETC, and its activity has been used as a quantitative marker for the presence of intact mitochondria (Abdul Razak et al. 2019). Aging affects citrate synthase activity in the brain as early as middle age as shown by previous studies that citrate synthase activity was declined in the brain of old mice (72 weeks) (Navarro et al. 2002) and certain brain regions (cerebral hemisphere, cerebellum, brain stem) of middle-aged rats (Sandhu & Kaur 2003). Our result is concordant with previous studies that citrate synthase activity was decreased in the hippocampus of middle-aged rats,

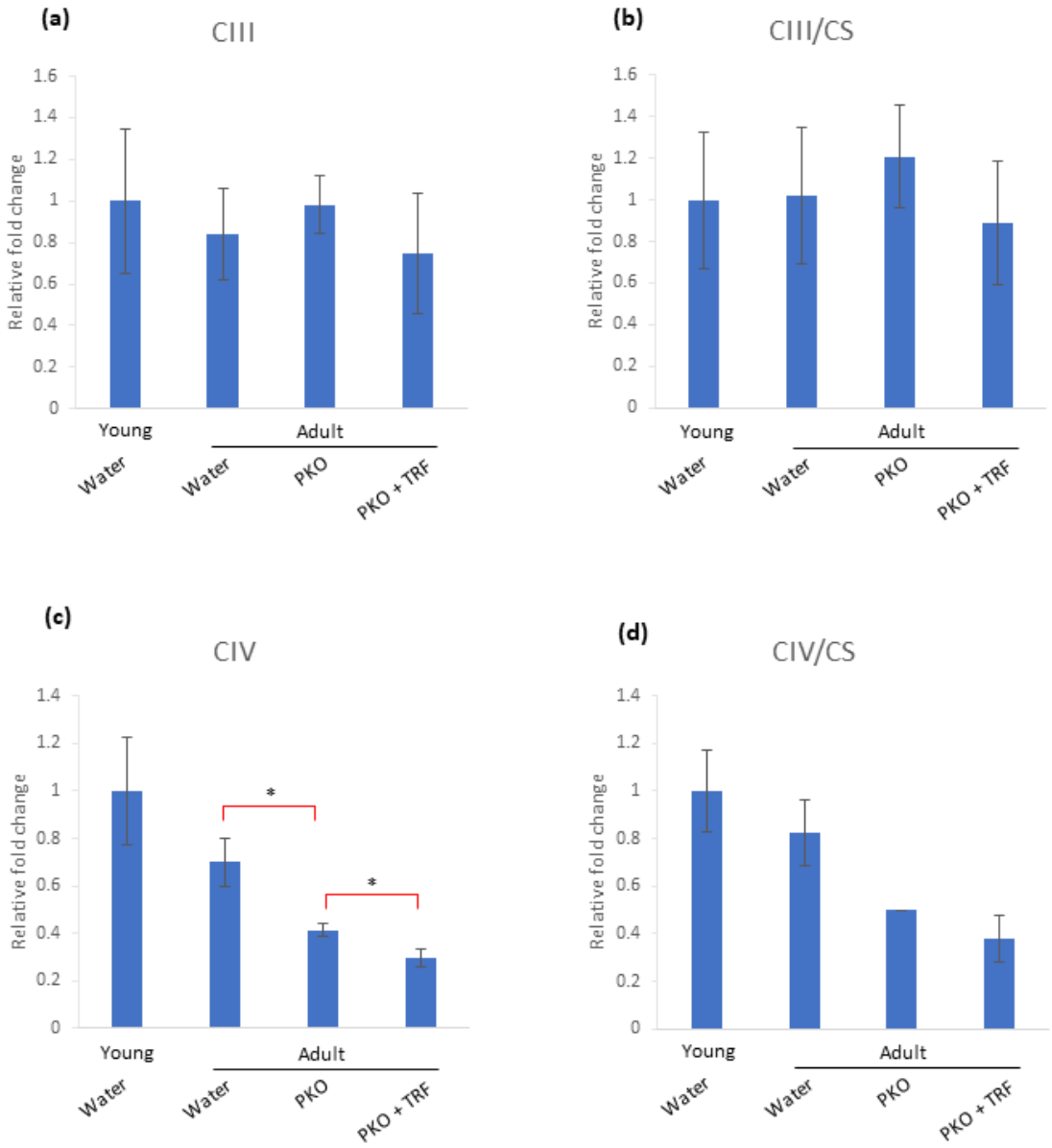

FIGURE 4. Complex III and IV activities: (a) The overall activity of complex III, (b) activity of complex III normalized with citrate synthase activity, (c) overall activity of complex IV, and (d) activity of complex IV normalized with citrate synthase activity in rat hippocampus. Young rats were supplemented with water, while adult rats were supplemented with water, PKO, or PKO+TRF. N = 3 biological replicates with 3 technical replicates each + SD; *p<0.05

implying that reduction of mitochondrial intactness and mass is apparent even at middle age. The progressive loss of mitochondrial intactness and mass at middle age might be implicated in age-associated neurodegenerative disorders as the hippocampus plays a central role in memory and learning processes (Abdul Razak et al. 2018).

Complex I deficiency is often encountered in aging and age-associated neurodegenerative disorders. Abnormal assembly of complex I has been postulated as the cause of complex I deficiency in Parkinson's disease (Pathak & Davey 2008). Complex I is regarded as the most likely ETC impairment site as many subunits of complex I are encoded by mitochondrial DNA

(Sandhu & Kaur 2003). Mitochondrial DNA is more susceptible to oxidative damage due to its proximity to the ROS production site and lack of efficient DNA repair mechanisms. The decline of complex I overall activity has been reported in the cortex, cerebellum, and brainstem of old mice (70 weeks) and mice of a neurodegenerative model (pcd5J) (Pollard et al. 2016). Our data show that complex I and I+III activities in overall and individual mitochondria of the hippocampus remained unchanged between young and middle-aged Sprague Dawley rats. Previous studies have reported that overall activity of complex I+III decreased in the hippocampus (Navarro et al. 2008) and certain brain regions (hemisphere, cerebellum, and diencephalon) of middle-aged Wistar rats (Sandhu & Kaur 2003). However, the complex I activity alone was not assessed in these studies. The contradiction findings could be attributed to different rat species used; Sprague Dawley rats seem to have more resistance to complex I deficiency in midlife, but whether the animal strains play a role in complex I deficiency during aging remains for further investigations.

Previous studies have reported that overall activity of complex II+III remained unchanged in the hippocampus of middle-aged rats (Navarro et al. 2011, 2008) and the brain of middle-aged and old mice (52 and 72 weeks, respectively). Another study has shown that overall activity of complex II+III decreased in certain brain regions (cerebral hemisphere, cerebellum, and brain stem) of middle-aged rats, but the decline of overall activity for complex II+III with age was less extensive than complex I+III and IV (Sandhu & Kaur 2003). Complex II+III activity is less affected with age, and this has been attributed to all subunits of complex II are encoded by nuclear DNA that are more resistant to oxidative stress. In contrast, our data show that complex II activity was increased with age in overall or individual mitochondria, while complex II+III activity remained unchanged. Elevation of complex II activity might indicate dysfunction of complex II which is related to the role of complex II as an enhancer of ROS generation. As ETC is directly linked to the citric acid cycle by complex II, high concentration of succinate induced reverse transfer of electron from complex II to I, and this process is accompanied by overproduction of superoxide, while inhibition of complex II reduced complex I-related ROS generation (Dröse 2013). The increase of complex II activity in adult rats compared with young rats of this study might indicate an age-associated dysfunction of complex II. Further studies are needed to assess the link between complex II activity and ROS production.

PKO used in this study was stripped off vitamin E and served as a vehicle for TRF. Interestingly, our findings indicate that PKO influenced the activities of mitochondrial respiratory chain complexes. As compared with control rats, PKO decreased activities of complex I and IV, while increased complex I+III activity in the hippocampus of adult rats. The findings remained the same for data normalized with citrate synthase activity except for complex IV activity. Our findings suggest that PKO without TRF might decrease the efficiency of ETC, especially on complex I activity. The increase of complex I+III activity by PKO could be attributed to the cellular compensation mechanism for the deficient complex I activity by enhancing of the coupled activity of complex I+III.

The effect of vitamin E members on mitochondrial respiratory chain complexes' activities was mainly determined using tocopherol and its derivative (Navarro et al. 2011, 2005). On the other hand, rice bran extract, which is rich in gamma-tocotrienol, alpha-tocopherol, and alpha-tocotrienol, was shown to increase citrate synthase activity in the brain of guinea pig (Hagl et al. 2013). However, our study found that TRF did not prevent the decline of citrate synthase activity in middle-aged rats' hippocampus. Much earlier supplementation might be needed to alleviate the loss of mitochondrial intactness and mass.

Our results show that TRF increased the overall activity of complex I as compared with PKO-treated adult rats. These findings imply that TRF restored the overall activity of complex I which was reduced by PKO. TRF might have a protective effect against age-associated neurodegenerative disorders with complex I deficiency in the brain. However, the gross increase of complex I activity by TRF was not significant after normalized with citrate synthase activity implying that the enhancement effect by TRF was not due to increased mitochondrial mass as there was no difference in citrate synthase activity between TRF- and PKO-treated adult rats rather might be attributed to increase of complex I activity by TRF in a certain population of mitochondria. Whether TRF selectively enhanced complex I activity in a specific population of mitochondria remains for further investigations. On the other hand, previous studies have shown the increment of overall activity for complex I+III in the hippocampus of middle-aged rats (Navarro et al. 2011) and the brain of aged mice (52 - 76 weeks) (Navarro et al. 2005) supplemented by alpha-tocopherol and alpha-tocopherol acetate, respectively. However, our results show that TRF did not affect the overall activity of complex I+III. TRF seems to be only modulating the

overall activity of complex I instead of the complex I+III. As previous studies used tocopherols instead of TRF, the different findings might have resulted from the distinct actions of tocotrienols and tocopherols.

The decline of complex IV overall activity has been reported in the hippocampus of middle-aged rats (Navarro et al. 2008), while supplementation of alpha-tocopherol and alpha-tocopherol acetate restored the overall activity of complex IV in the hippocampus of middle-aged rats (Navarro et al. 2011) and the brain of a senescence-accelerated mouse model (CD-1/UCadiz) (Navarro et al. 2005), respectively. In contrast to the previous findings, our data show that TRF decreased the overall activity of complex IV in middle-aged rats' hippocampus. The decline of complex IV activity with age might reflect the reduction of OXPHOS capacity as complex IV is the last electron acceptor of the respiratory chain. However, another study has suggested that the reduction of complex IV activity might have a beneficial effect. Loss of complex IV assembly protein, SURF1, dramatically decreased complex IV activity in the major organs, including the brain, while increasing mice's lifespan (Dell'Agnello et al. 2007). These findings imply the link of longevity with mitochondrial bioenergetics. TRF has been shown to possess an anti-senescent effect on human diploid fibroblasts (Makpol et al. 2011), reverse muscle cell aging (Khor et al. 2016; Lim et al. 2013), restore lifespan of C. elegans under oxidative insult (Goon et al. 2013), and prolong the lifespan of S. cerevisiae (Tajul Arifin et al. 2019). The anti-aging effect of TRF may be extended to rats through complex IV, but the implication of reduced complex IV activity by TRF on the lifespan of rats remains to be further investigated. The effects of age, PKO, and TRF on activities of mitochondrial respiratory chain complexes and citrate synthase were summarized in Figure 5.

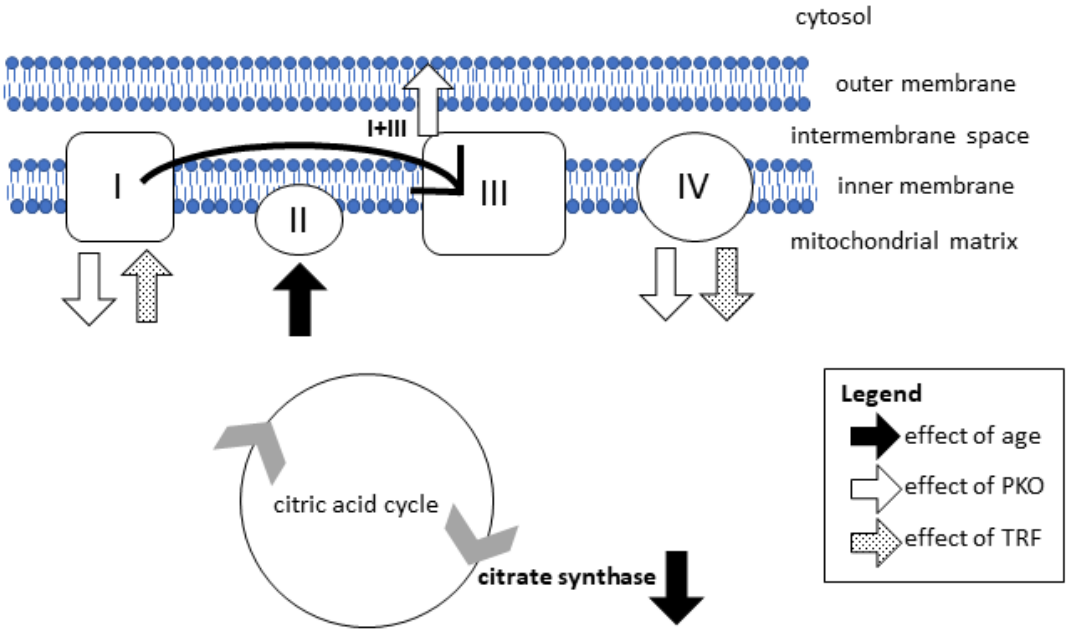

FIGURE 5. The effects of age, PKO, and TRF on activities of mitochondrial respiratory chain complexes and citrate synthase. Citrate synthase is part of the citric acid cycle. The upward arrow indicates increased enzymatic activity, while the downward arrow indicates decreased enzymatic activity

CONCLUSION

This study shows that mitochondrial mass and intactness of the rat's hippocampus were reduced at middle age. PKO had modulatory effects on complex I, I+III, and IV activities of adult rats. TRF restored the overall activity of complex I in PKO-treated adult rats. Mitochondrial dysfunction in the brain is apparent at midlife, while the reservation of respiratory chain capacity by TRF might have a protective effect against neurodegeneration especially those associated with complex I deficiency.

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