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High-Fat Diet and Voluntary Chronic Aerobic Exercise Recover Altered Levels of Aging-Related Tryptophan Metabolites along the Kynurenine Pathway

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Tryptophan metabolites regulate a variety of physiological processes, and their downstream metabolites enter the kynurenine pathway. Age-related changes of metabolites and activities of associated enzymes in this pathway are suggestable and would be potential intervention targets. Blood levels of serum tryptophan metabolites in C57BL/6 mice of different ages, ranging from 6 weeks to 10 months, were assessed using high-performance liquid chromatography, and the enzyme activities for each metabolic step were estimated using the ratio of appropriate metabolite levels. Mice were subjected to voluntary chronic aerobic exercise or high-fat diet to assess their ability to rescue age-related alterations in the kynurenine pathway. The ratio of serum kynurenic acid (KYNA) to 3-hydroxylkynurenine (3-HK) decreased with advancing age. Voluntary chronic aerobic exercise and high-fat diet rescued the decreased KYNA/3-HK ratio in the 6-month-old and 8-month-old mice groups. Tryptophan metabolites and their associated enzyme activities were significantly altered during aging, and the KYNA/3-HK ratio was a meaningful indicator of aging. Exercise and high-fat diet could potentially recover the reduction of the KYNA/3-HK ratio in the elderly.

Key words: tryptophan metabolites, kynurenine pathway, aging, voluntary chronic aerobic exercise, high-fat diet

INTRODUCTION

As the average human lifespan is extended, the global population age is increasing, and investigation into the mechanisms of biological aging is becoming important. However, appropriate biomarkers of aging are difficult to discover due to the complexity and ambiguous causal relationships of various phenotypes related to the aging process. Although this is an area of active study, biomarkers that accurately represent the internal aging process remain elusive [1, 2].

Tryptophan (TRP) is a precursor of the neurotransmitter serotonin and melatonin [3]. TRP is metabolized into kynurenine (KYN) by tryptophan 2,3-dioxygenase (TDO) or indoleamine

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2,3 dioxygenase (IDO) and then enters into the kynurenine pathway. This kynurenine pathway, produces NAD+, kynurenic acid (KYNA), and xanthurenic acid [4]. KYN is metabolized into the neuroprotective kynurenic acid by kynurenine aminotransferase (KAT), or into 3-hydroxyl(OH)-kynurenine (3-HK), an intermediate metabolite with neurotoxic properties, by kynurenine 3 monooxygenase (KMO) [4, 5].

Alteration of the kynurenine pathway has been described in various diseases such as ischemic stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease, and multiple sclerosis [4-6]. Recent studies have focused on identifying underlying disease mechanisms and their potential targets for therapeutic intervention [4, 6, 7]. KYN metabolite levels have represented as the overall status of the central nervous system (CNS) [5], and measuring TRP metabolites could be a useful biomarker of CNS aging. In addition, aerobic exercise or high-fat diet have been proposed to recover somatic alterations by aging and cognitive decline [8-13].

Based on the idea of TRP metabolites as a biomarker of aging, we examined whether there are differences in TRP metabolites and enzyme activity of the kynurenine pathway according to ages. In addition, we also attempted to evaluate whether the TRP metabolites can be modified by aerobic exercise or high-fat diet.

MATERIALS AND METHODS

Mice

C57BL/6 (Orient Bio Inc) mice were used for all experiments. Mice were housed three mice per each cage (for 8-month-old mice, two mice per cage after day 5) under pathogen-free conditions with a 12/12 hour light-dark cycle and ad-libitum access to water and food. All animal protocols were approved by the Institutional Animal Care and Use Committee of Seoul National University Clinical Research Institute.

Aging, diet, and exercise assays

6 weeks old male C57BL/6 mice were arranged into 5 groups (total n=29) and were sacrificed at different ages; 6 weeks, 3 months, 6 months, 8 months (n=6 per timepoint) or 10 months (n=5). To evaluate the effect of a high-fat diet and exercise, separate groups of mice at the age of 2, 5, and 7 months (total n=57) were arranged into a high-fat diet group (n=5 per timepoint), an exercise group (n=5 per timepoint), or a control group (n=9 for 2 months and 5 month, n=10 for 7 months). Mice in the high-fat diet group were fed a high-fat diet (D12492, Research Diets, Inc., New Brunswick, NJ) for 1 month, which is consisted of 20% protein, 20% carbohydrate, and 60% fat (in kcal%). Fat composing the diet was formulated with soybean oil and lard. Mice in the exercise group were given voluntary chronic aerobic exercise for 1 month by providing a running wheel (Lafayette Instument, Co., Lafayette, IN) in the cage [14, 15]. Numbers of revolutions were measured automatically by the rotation counter within the wheel and the length of exercise was calculated (0.4 meters per revolution). For the control group, mice were fed an ordinary diet (PicoLab® Rodent Diet 20, LabDiet, St. Louis, MO), consisted of 20% protein, 52.9% carbohydrate, and 10.6% fat (in kcal%), and without a running wheel in the cage. For each mouse, blood samples were taken at the time of sacrifice (3, 6, and 8 months) to quantify TRP metabolite levels after overnight starvation.

Measurement of tryptophan metabolites and enzyme activity

The concentration of TRP and its metabolites (KYN, KYNA, and 3-HK) in serum were determined using a modified version of a previously established method [16, 17]. Briefly, sample protein was precipitated using methanol. Tryptophan methyl ester was used as an internal standard for the quantification of TRP and the above metabolites. A LC-MS/MS system with an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled to an Applied Biosystems API4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) was used for quantification. Chromatographic separation was conducted on a Synergi Polar-RP (Phenomenex Inc., Torrance, CA, USA) with a mobile phase consisting of 5 mM ammonium formate in distilled water and 0.1% formic acid in methanol. The intra- and inter-day accuracies of this method ranged from 99.76% to 106.8%, and the intra- and inter-day precisions were greater than 5.4% throughout.

To estimate enzyme activity of the TRP metabolic pathway, a conversion ratio between two of the metabolites was quantified. Activity of TDO and IDO was estimated by calculating the ratio of serum KYN to TRP levels (KYN concentration divided by TRP concentration) [18, 19]. KMO activity was estimated using the ratio of 3-HK to KYN levels (3-HK concentration divided by KYN concentration), and KAT activity using the ratio of KYNA and KYN levels (KYNA concentration divided by KYN concentration). The ratio of KYNA and 3-HK, which represents the ratio of KAT activity and KMO activity. was also calculated.

Statistical analysis

SPSS for Windows (version 18.0; SPSS, Chicago, IL) was used for all statistical analysis. Data are presented as median and interquartile range. A Mann-Whitney U-test and a Kruskal-Wallis test were used for comparisons of TRP metabolites and their ratios among animal groups. Student's t-test was used for comparison of exercise amounts. Statistical significance was considered at $p<0.05$.

RESULTS

The effects of aging on tryptophan metabolites and enzyme activity

Among the age groups, there were significant differences in the levels of serum TRP metabolites (Table 1 and Fig. 1), specifically in TRP (p=0.039), KYN (p=0.013), and KYNA (p=0.01) concentrations. These metabolites showed more reduced levels in the older groups. However, 3-HK concentration did not differ significantly between the age groups (p=0.269). KYN/TRP ratios were significantly different, with the lowest level at 6 months (p=0.015). In addition, the level of KYNA/KYN ratio (p=0.001) and the KYNA/3-HK ratio (p=0.001) were significantly reduced in the older groups compared with the younger groups. However, there were no significant differences detected in 3-HK/KYN ratio (p=0.067).

The effects of high-fat diet on tryptophan metabolites and enzyme activity

In the high-fat diet group, TRP concentration was higher in 8-month-old mice (p=0.002), while the concentration of KYN was lower in the 3-month-old mice compared with control groups (p=0.003) (Table 2 and Fig. 2). 3-HK was decreased in

Table 1. Serum tryptophan metabolites and enzyme activities in C57BL/6 mice with different age groups

	6 weeks (n=6)	3 months (n=6)	6 months (n=6)	8 months (n=6)	10 months (n=5)	p-value
TRP (μM)	66.25 (59.36-77.07)	71.85 (52.29-75.83)	68.22 (57.97-78.57)	48.66 (43.40-55.74)	52.15 (47.02-63.17)	0.039
KYN (nM)	1233.56 (1111.63-1294.89)	702.18 (601.59-1039.49)	717.33 (575.21-829.58)	636.62 (493.41-934.99)	902.22 (731.32-1007.00)	0.013
3-HK (nM)	210.28 (157.38-250.30)	199.97 (136.19-328.48)	139.03 (120.44-169.85)	165.78 (130.57-231.37)	142.31 (110.45-227.48)	0.269
KYNA (nM)	125.81 (86.43-184.72)	67.31 (53.70-96.64)	30.32 (24.65-39.91)	36.18 (20.13-50.27)	37.49 (26.74-64.38)	0.001
KYN/TRP ratio	0.017 (0.015-0.020)	0.012 (0.009-0.014)	0.012 (0.009-0.012)	0.013 (0.010-0.019)	0.015 (0.013-0.020)	0.015
3-HK/KYN ratio	0.18 (0.16-0.21)	0.31 (0.21-0.33)	0.19 (0.18-0.26)	0.29 (0.24-0.33)	0.16 (0.13-0.27)	0.067
KYNA/KYN ratio	0.088 (0.079-0.135)	0.087 (0.075-0.100)	0.043 (0.027-0.057)	0.040 (0.037-0.070)	0.041 (0.033-0.061)	0.001
KYNA/3-HK ratio	0.48 (0.36-0.83)	0.35 (0.23-0.41)	0.18 (0.13-0.27)	0.18 (0.12-0.24)	0.28 (0.17-0.33)	0.002

Data presented as median and interquartile range. TRP indicates tryptophan; KYN, kynurenine; 3-HK, 3-hydroxylkynurenine; KYNA, kynurenic acid. p-values were provided by Kruskal-Wallis test.

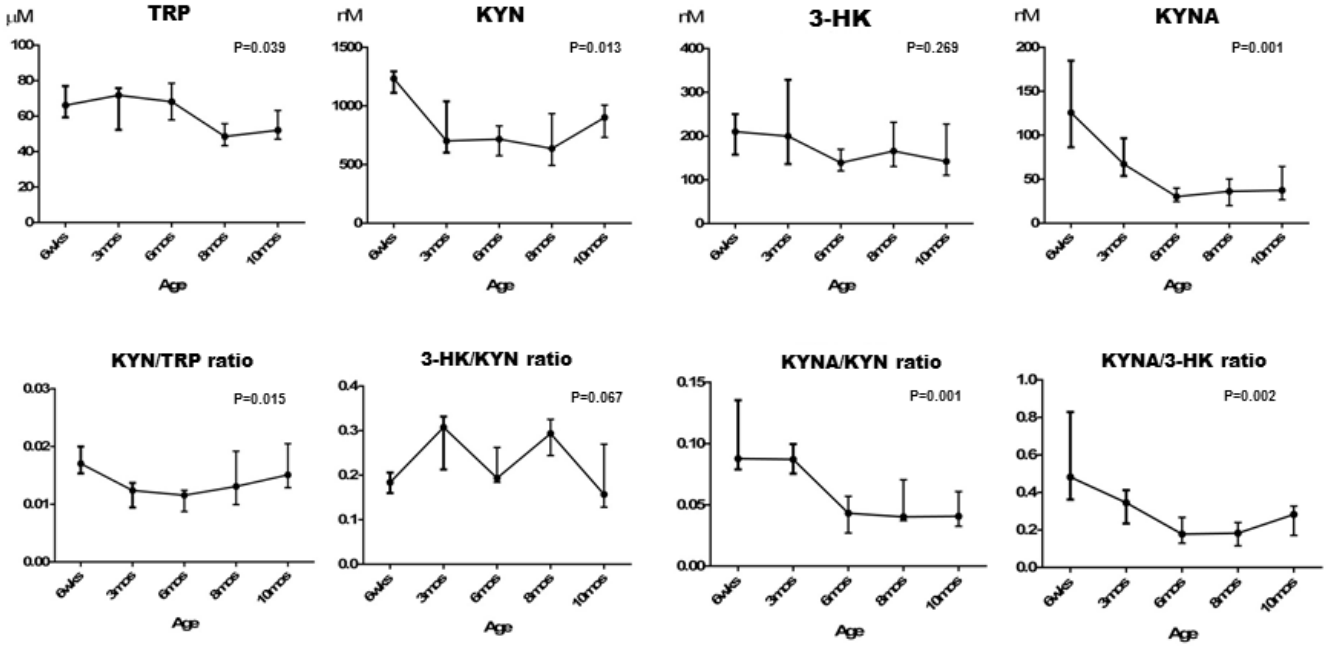

Fig. 1. The change of serum tryptophan metabolites and enzyme activities during aging. Tryptophan metabolites (tryptophan [TRP], kynurenine [KYN], kynurenic acid [KYNA], 3-OH-kynurenine [3-HK]) and their ratios (KYN/TRP ratio, 3-HK/KYN ratio, KYNA/KYN ratio and KYNA/3-HK ratio) in C57BL/6 mice aged 6 weeks, 3 months, 6 months, 8 months (n=6 per timepoint), and 10 months (n=5). Data presented as median and interquartile range. p-values were provided by Kruskal-Wallis test.

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Table 2. Tryptophan metabolites and enzyme activities in C57BL/6 mice of 3-month-old, 6-month-old and 8-month-old which are fed a high-fat diet for previous 1 month and fed an ordinary diet in control group

	3 months			6 months			8 months		
	Control (n=9)	High-fat diet (n=5)	p-value	Control (n=9)	High-fat diet (n=5)	p-value	Control (n=10)	High-fat diet (n=5)	p-value
TRP (μM)	73.21 (52.42-79.91)	49.78 (47.30-58.00)	0.072	61.30 (48.96-72.92)	67.63 (54.53-77.50)	0.549	48.66 (46.96-55.78)	83.17 (77.93-87.97)	0.002**
KYN (nM)	721.85 (661.10-962.23)	418.46 (399.40-452.64)	0.003**	709.85 (575.13-850.09)	681.99 (628.92-719.93)	0.841	527.34 (479.62-791.97)	578.73 (545.83-1160.83)	0.178
3-HK (nM)	237.04 (132.26-341.89)	104.83 (91.27-116.19)	0.028*	174.50 (152.72-239.92)	106.81 (85.02-150.69)	0.096	176.22 (131.66-252.39)	76.49 (64.07-120.21)	0.221
KYNA (nM)	66.54 (49.98-94.44)	47.14 (35.79-58.94)	0.072	32.69 (22.90-47.86)	46.65 (41.32-63.44)	0.021*	39.12 (24.80-59.20)	48.70 (44.13-57.49)	0.028*
KYN/TRP ratio	0.012 (0.009-0.014)	0.008 (0.008-0.009)	0.006**	0.012 (0.010-0.013)	0.010 (0.008-0.013)	0.257	0.010 (0.009-0.015)	0.007 (0.006-0.014)	0.221
3-HK/KYN ratio	0.34 (0.20-0.37)	0.24 (0.21-0.27)	0.162	0.22 (0.21-0.37)	0.16 (0.12-0.20)	0.053	0.31 (0.27-0.36)	0.09 (0.08-0.11)	0.903
KYNA/KYN ratio	0.083 (0.072-0.114)	0.104 (0.088-0.137)	0.257	0.049 (0.030-0.066)	0.070 (0.061-0.094)	0.045*	0.066 (0.036-0.097)	0.074 (0.052-0.086)	0.011*
KYNA/3-HK ratio	0.31 (0.21-0.54)	0.45 (0.39-0.51)	0.257	0.21 (0.13-0.25)	0.45 (0.33-0.67)	0.005**	0.21 (0.12-0.34)	0.70 (0.51-0.74)	0.018*

Data presented as median and interquartile range. TRP indicates tryptophan; KYN, kynurenine; 3-HK, 3-hydroxylkynurenine; KYNA, kynurenic acid. p-values were provided by Mann-Whitney U test. *p < 0.05, **p < 0.01.

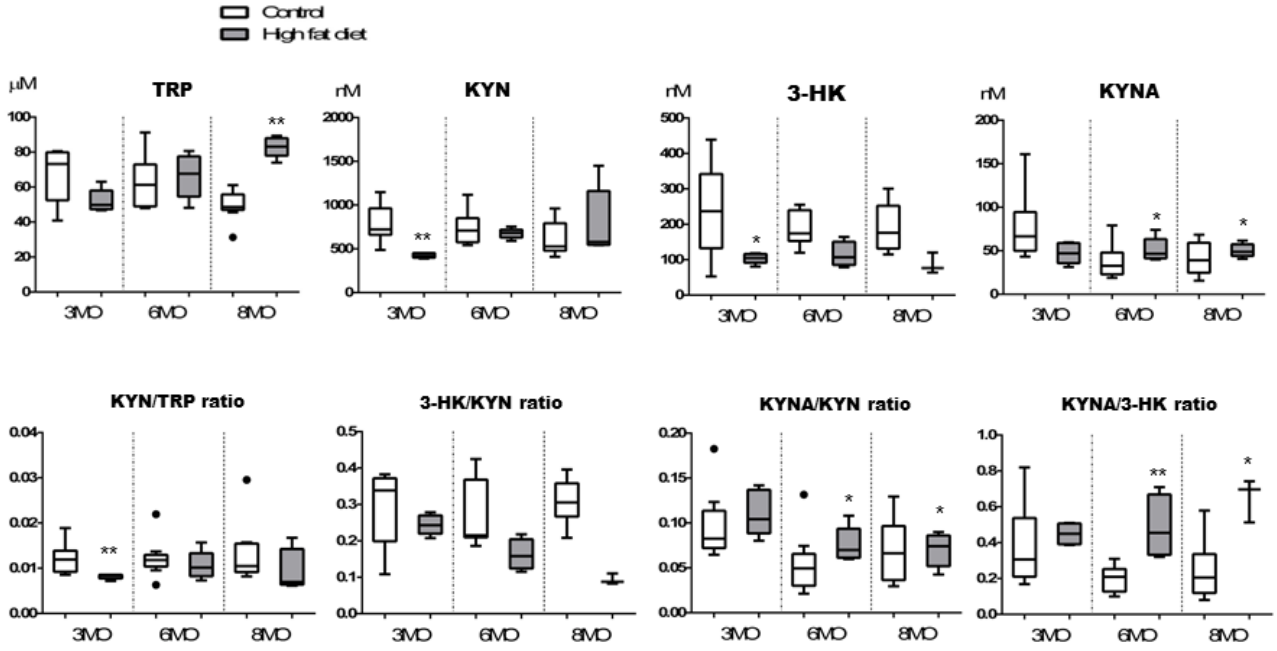

Fig. 2. The effect of high-fat diet on serum tryptophan metabolites and enzyme activities. Tryptophan metabolites (tryptophan [TRP], kynurenine [KYN], kynurenic acid [KYNA], 3-OH-kynurenine [3-HK]) and their ratios (KYN/TRP ratio, 3-HK/KYN ratio, KYNA/KYN ratio and KYNA/3-HK ratio) in C57BL/6 mice of 3-month-old, 6-month-old and 8-month-old (n=5 per timepoint) which are fed a high-fat diet for previous 1 month and fed an ordinary diet in control group (n=9 in 3-month-old and 6-month-old, and n=10 in 8-month-old). The black horizontal line in each box represents the median, with the boxes representing the interquartile range. *p<0.05, **p<0.01 (Mann-Whitney U test).

3-month-old group compared with controls (p=0.028), while KYNA increased in the 6-month-old (p=0.021) and 8-month-old group (p=0.028) (Fig. 2). The KYN/TRP ratio was decreased in the 3-month-old mice compared with controls (p=0.006), while the 3-HK/KYN ratio was constant throughout the age groups. KYNA/KYN ratio was increased in the 6-month-old (p=0.045)

and 8-month-old age group (p=0.011), resulting in the elevation of KYNA/3-HK ratio in the 6-month-old and 8-month-old mice (p=0.005 and p=0.018, 6-month-old and 8-month-old, respectively) (Fig. 2).

The effects of voluntary chronic aerobic exercise on tryptophan metabolites and enzyme activity

The number of revolution in the voluntary chronic aerobic exercise group was 8346.52±2575.11, 8522.94±1696.03 and 8896.21±4372.32 revolutions/mouse·day in 3-, 6-, and 8-month-old mice, respectively which could be converted into the mean length of wheel running of 3338.61±1030.04, 3409.14±678.41 and 3558.48±1748.93 meters/mouse·day. There were no differences of the exercise amount between the age groups (p=0.781). In the exercise group, TRP concentration in 8-month-old mice (p=0.002), and KYN concentration in 6-month-old mice (p=0.014) were increased compared with control groups (Table 3 and Fig. 3). 3-HK concentration in the 6-month-old mice (p=0.021), and the level of KYNA in 6-month-old 8-month-old mice (p=0.003 and p=0.007, 6-month-old and 8-month-old, respectively) was significantly elevated. The KYN/TRP ratio was elevated in the 6-month-old mice mice (p=0.020), while the 3-HK/KYN ratio was decreased in the 3-month-old and 8-month-old mice (p=0.039 and p=0.028, 3-month-old and 8-month-old respectively). KYNA/KYN ratio (p=0.004 and p=0.016, 6-month-old and 8-month-old respectively) and the KYNA/3-HK ratio (p=0.005 and p=0.032, 6-month-old and 8-month-old respectively) were both elevated in 6-month-old 8-month-old mice.

DISCUSSION

Our data suggest that serum TRP metabolites and the activity of enzymes in the kynurenine pathway are altered by aging and are further affected by diet or exercise. The KYNA/3-HK ratio represents the overall status of the kynurenine pathway, and alterations in this ratio are strongly related to aging. There was also an age-related increase in the neurotoxic products compared with neuroprotective products. These products signify the relative neurotoxic and neuroprotective processes downstream of the kynurenine pathway and are potential biomarkers and therapeutic targets of aging. On the other hand, exposure to a high-fat diet or exercise recovered these changes by raising the activity of KYNA/3-HK ratio. These effects were shown in only certain age groups, which implies age specific intervention via diet or exercise might be beneficial.

Two products of KYN metabolism, 3-HK and KYNA, are produced through separate metabolic pathways and have distinct effects. The effects of 3-HK are toxic, as it promotes the production of reactive oxygen species [20]. In contrast, KYNA has a neuroprotective effect through inhibition of N-methyl-D-aspartate (NMDA) [21], glutamate [22], kainate [23], and α7 nicotinic ace-

Table 3. Tryptophan metabolites and enzyme activities in C57BL/6 mice of 3-month-old, 6-month-old and 8-month-old subjected to voluntary chronic aerobic exercise by inserting a running wheel in the cage for previous 1 month and without running wheel in control group

	3 months			6 months			8 months		
	Control (n=9)	Exercise (n=5)	p-value	Control (n=9)	Exercise (n=5)	p-value	Control (n=10)	Exercise (n=5)	p-value
TRP (μM)	73.21 (52.42-79.91)	59.51 (53.72-68.31)	0.205	61.30 (48.96-72.92)	67.92 (58.10-73.09)	0.386	48.66 (46.96-55.78)	65.44 (62.97-80.75)	0.002**
KYN (nM)	721.85 (661.10-962.23)	752.11 (641.41-855.37)	0.947	709.85 (575.13-850.09)	1026.35 (1021.55-1200.21)	0.014*	527.34 (479.62-791.97)	704.56 (634.68-861.85)	0.178
3-HK (nM)	237.04 (132.26-341.89)	139.08 (94.15-156.26)	0.096	174.50 (152.72-239.92)	305.73 (246.41-351.50)	0.021*	176.22 (131.66-252.39)	123.01 (95.79-160.28)	0.176
KYNA (nM)	66.54 (49.98-94.44)	68.55 (50.95-90.74)	1.000	32.69 (22.90-47.86)	148.57 (126.31-189.29)	0.003**	39.12 (24.80-59.20)	88.73 (68.06-108.67)	0.007**
KYN/TRP ratio	0.012 (0.009-0.014)	0.013 (0.011-0.014)	0.641	0.012 (0.010-0.013)	0.016 (0.014-0.020)	0.020*	0.010 (0.009-0.015)	0.011 (0.009-0.012)	0.624
3-HK/KYN ratio	0.34 (0.20-0.37)	0.18 (0.14-0.18)	0.039*	0.22 (0.21-0.37)	0.29 (0.24-0.34)	0.280	0.31 (0.27-0.36)	0.15 (0.10-0.23)	0.028*
KYNA/KYN ratio	0.083 (0.072-0.114)	0.083 (0.069-0.112)	0.877	0.049 (0.030-0.066)	0.145 (0.111-0.181)	0.004**	0.066 (0.036-0.097)	0.128 (0.101-0.155)	0.016*
KYNA/3-HK ratio	0.31 (0.21-0.54)	0.46 (0.38-0.62)	0.217	0.21 (0.13-0.25)	0.56 (0.45-0.70)	0.005**	0.21 (0.12-0.34)	0.66 (0.47-0.58)	0.032*

Data presented as median and interquartile range. TRP indicates tryptophan; KYN, kynurenine; 3-HK, 3-hydroxylkynurenine; KYNA, kynurenic acid. p-values were provided by Mann-Whitney U test. *$p < 0.05$, **$p < 0.01$.

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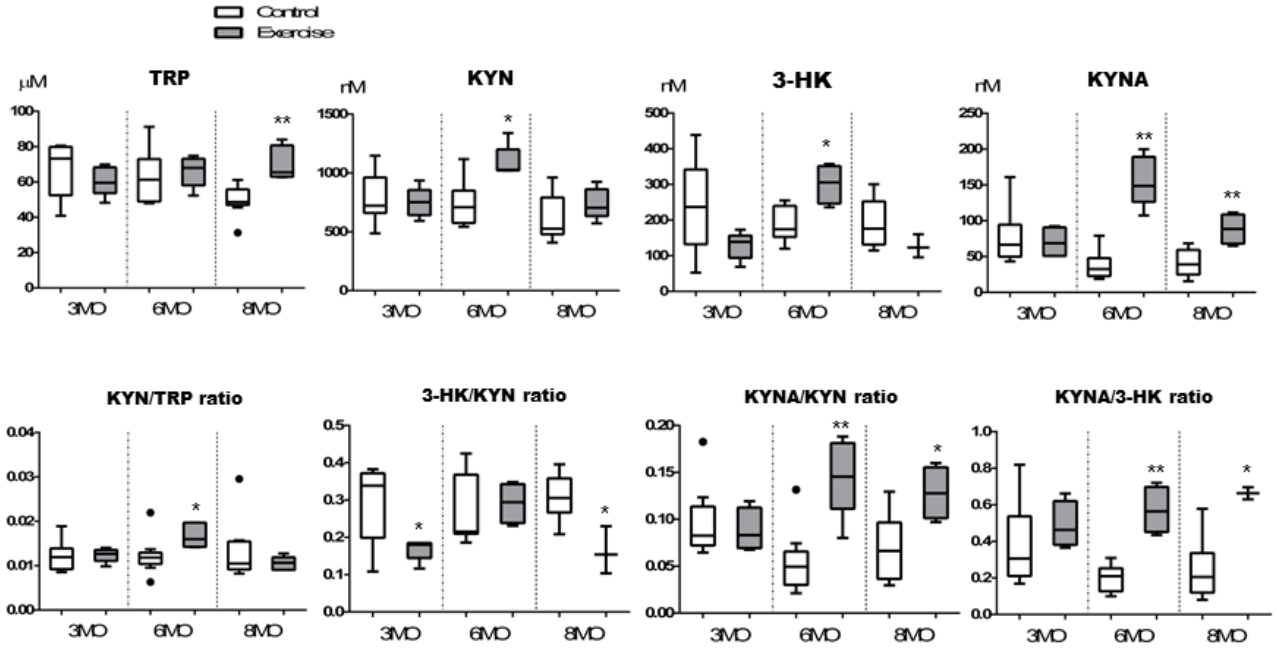

Fig. 3. The effect of voluntary chronic aerobic exercise on serum tryptophan metabolites and enzyme activities. Tryptophan metabolites (tryptophan [TRP], kynurenine [KYN], kynurenic acid [KYNA], 3-OH-kynurenine [3-HK]) and their ratios (KYN/TRP ratio, 3-HK/KYN ratio, KYNA/KYN ratio and KYNA/3-HK ratio) in C57BL/6 mice of 3-month-old, 6-month-old and 8-month-old (n=5 per timepoint) subjected to voluntary chronic aerobic exercise by inserting a running wheel in the cage for previous 1 month and without running wheel in control group (n=9 in 3-month-old and 6-month-old, and n=10 in 8-month-old). The black horizontal line in each box represents the median, with the boxes representing the interquartile range. *p<0.05, **p<0.01 (Mann-Whitney U test).

tylcholine receptors [24], as well as potent antioxidant properties [25]. Multiple inflammatory conditions activate IDO, including infection [26], cancer [27], atherosclerosis [28], obesity [29], and chronic heart disease [30]. For these conditions, IDO is considered to have anti-inflammatory effects [7]. The activities of KAT and KMO directly contribute to the production of KYNA and 3-HK, respectively. Therefore, these enzymes have a crucial role in this pathway and are potential targets for pharmacological intervention [6, 7, 31].

Several animal studies suggest TRP metabolism has a role in the aging process [32]. There are previous studies that demonstrated the changes of plasma TRP level in human, mostly showing a decline with aging [33-35]. TDO and IDO activity decrease with aging in most organs while IDO activity in brain increase. Mild inflammatory environment related to aging process might explain IDO activation [7]. Thus, decreased KYN/TRP ratio compared to the youngest age group and mild elevation during aging in our results might be explained by both decreased TDO and IDO activity in older age groups and decline of TRP level and IDO activation by inflammatory processes during aging. Aside from KYN/TRP ratio, we have demonstrated that there is a decrease in KYNA, KYNA/KYN ratio, and the KYNA/3-HK ratio during aging, suggesting a reduced neuroprotective capacity as aging proceeds. On the other hand, 3-HK concentration and 3-HK/KYN ratio remained constant throughout the aging process, suggesting the neurotoxic properties of this pathway do not change as aging proceeds. In addition, senile blood-brain-barrier dysfunction could increase transmission of the TRP metabolites that do not usually enter the CNS, such as KYN and quinolinic acid. These metabolites might further contribute to neurological degenerative disorders and cognitive decline related to aging process [36, 37]. Thus, although our study only demonstrated the changes of TRP metabolites in the blood, there might be a possibility that these can influence the age-related changes of the brain. Further study focused on age-related TRP metabolite level changes directly measured in the brain along with behavior studies for cognition and memory might be helpful to reveal the potential linkage.

A high-fat diet and exercise increased the KYNA/3-HK ratio in the 6-month-old and 8-month-old mice. However, these changes were not observed in the 3-month-old mice. This increase in the KYNA/3-HK ratio appeared to be the result of distinct processes as both elevated KYNA/KYN ratio and decreased 3-HK/KYN ratio were observed in the exercise group, while a decrease in the 3-HK/KYN ratio seems to mainly contribute to the increase of KYNA/3-HK ratio in the high-fat diet group. Exercise is known to accelerate TRP metabolism through activation of IDO [38]. One

previous study showed that wheel running in rat increases plasma and brain free TRP level via stimulating lipolysis, and unesterified fatty acids decreases TRP binding to albumin [39]. A recent animal study indicates that exercise increases the skeletal expression of KAT through the PGC-1 α1-PPAR α/δ pathway [40], which could be a potential mechanism for explaining our results. In addition, our data show a reduced 3-HK/KYN ratio, suggesting another beneficial result of exercise is reducing oxidative stress.

The effect of a high-fat diet on the kynurenine pathway is not well understood. Previous study in rats revealed inhibition of liver TDO by high-fat-diet, increase in plasma and brain free TRP level and lowering total TRP level [41]. Another previous work in rabbits detected no changes in enzyme activity in this pathway by a high-fat diet [42]. While exercise has well-characterized beneficial effects [8, 9, 11], a high-fat diet is known to negatively affect life span [11] and cognitive function [12, 43]. The proposed mechanisms contributing to the harmful effects of a high-fat diet include induction of oxidative stress, inflammation, insulin resistance, and a decrease in the expression of neurotrophic factors [11, 12, 43]. However, recent studies indicate an ameliorating effect of a high-fat diet on premature aging and neuronal damage [10, 44]. Increased NAD+ and sirtuin activity are proposed to be key mechanisms that lead to the beneficial effects on mitochondrial homeostasis [10, 45]. These inconsistencies could be the result of age-specific effects of a high-fat diet, as the rescuing effect of the kynurenine pathway only occurred in the 6-month-old and 8-month-old mice. Since NAD+ is one of the known end products of the kynurenine pathway [4], a link between kynurenine pathway and mitochondrial homeostasis might be suggested as the mechanism of the observed beneficial effect of high-fat-diet. Moreover a recent animal study, which revealed the association between gene expression and lifespan showed lifespan correlated with inflammation, apoptosis, PPAR signaling and various metabolic pathways [11], which are known to be also related with the kynurenine pathway. However, more specified studies are required for revealing the connection between interventions and beneficial effect in specific age groups.

Our study has several limitations. First, mice of the older age could be more pertinent to identify the effect of aging. However, in our study, mice older than 10-month-old showed poor compliance to voluntary exercise and diet, and were therefore excluded. Second, the effect of diet would have been verified more precisely by comparing the amount of diet consumption or body weight between mice receiving high-fat-diet and ordinal diet. A previous study demonstrated that animals receiving high-fat-diet showed increased body weight and more fat in body content [11]. Third, downstream effect should have been documented more directly by measuring metabolite levels in target organs or measuring concentration of downstream molecules such as NADPH.

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