# Increased Blood Quinolinic Acid after Exercise in Mice: Implications for Sensation of Fatigue after Exercise

# Yasuo Morimoto,\* Qian Zhang, and Koji Adachi

Kampo Research Laboratories, Kracie Pharma, Ltd., 3–1 Kanebo-machi, Takaoka, Toyama 933–0856, Japan (Received April 11, 2011; Accepted May 16, 2011;

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We have previously hypothesized that mental fatigue is caused by neuronal brain damage through the activation of N-methyl-D-aspartate receptors by quinolinic acid (QUIN). QUIN is a metabolite of tryptophan in the kynurenine pathway; this pathway is stimulated by several cytokines, including tumor necrosis factor (TNF)- $\alpha$ . Recently, we proved this mental-fatigue hypothesis by studying stress-loaded and lipopolysaccharide-treated mice. In the present study, we measured blood QUIN levels after exercise in mice to investigate whether QUIN also participates in causing the sensation of fatigue after exercise. In a weight-loaded swimming test, steel wires weighing about 5% of body weight were attached to the tails of mice that were then forced to swim until exhaustion. The serum QUIN levels of swimming mice were significantly higher than those of non-swimming mice. The serum TNF- $\alpha$  levels were also increased in swimming mice compared with nonswimming mice, although this effect was not significant. In a treadmill-running test, mice were forced to run for 150 min on a 10-degree uphill incline. The serum levels of both QUIN and TNF- $\alpha$  were significantly higher in treadmill-running mice than in nonrunning mice. Wheel-running counts, which reflect mental activity, were also measured in a running wheel-equipped home cage. Wheel-running counts of treadmill-running mice were significantly reduced compared with those of non-running mice. These results suggest that blood QUIN levels are increased after exercise and that this effect occurs through

enhanced tryptophan metabolism in the kynurenine

- Research Letter -

pathway due to TNF- $\alpha$  production. It is implied that QUIN participates in the sensation of fatigue after exercise.

Key words — quinolinic acid, fatigue, exercise, tumor necrosis factor- $\alpha$ , *N*-methyl-D-aspartate receptor

# INTRODUCTION

Fatigue is roughly classified into physical and mental fatigue. Mental fatigue is generally appeared as symptoms such as lassitude, sleepiness and decreased motivation without physical performance. The detailed mechanisms underlying mental fatigue have not been clarified, although various psychological factors such as stress or insomnia participate in it. We have previously hypothesized that mental fatigue is caused by neuronal brain damage through the activation of N-methyl-D-aspartate (NMDA) receptors by quinolinic acid (QUIN), one of the metabolites of tryptophan (TRP) in the kynurenine pathway. Recently, we found support for this hypothesis, at least in part, by studying stress-loaded and lipopolysaccharide (LPS)-treated mice. $^{1)}$ 

For physical fatigue, it is generally accepted that muscle fatigue mainly arises in muscle tissue from a disorder of energy metabolism, not limited to lactic acid accumulation, that is caused by long-duration or intense exercise.<sup>2,3)</sup> In addition to this muscle fatigue, the sensation of fatigue, which partly resembles mental fatigue in showing lassitude, sleepiness and decreased motivation, often develops after exercise. There are several reports to date that serotonin, a metabolite of TRP in the serotonin pathway, participates in fatigue after exercise.<sup>4, 5)</sup> We hypothesized that the sensation of fatigue after exercise is also caused by QUIN through the same mechanism described above for mental fatigue. To test this hypothesis, we investigated the influence of exercise on blood QUIN levels in mice.

### MATERIALS AND METHODS

Animals — Male, 5-week-old ddY mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). The animals were used in experiments after

<sup>\*</sup>To whom correspondence should be addressed: Kampo Research Laboratories, Kracie Pharma, Ltd., 3–1 Kanebo-machi, Takaoka, Toyama 933–0856, Japan. Tel.: +81-766-28-7953; Fax: +81-766-28-7960; E-mail: morimoto@phm.kracie.co.jp



Fig. 1. Influence of Weight-loaded Swimming on the Blood QUIN and TNF- $\alpha$  Levels in Mice

Steel wires that weighed 5% of body weight plus 0.4 g were attached to the tails of mice. Mice were forced to swim in a cylindrical acrylic tank (20 cm diameter) filled with 23°C water to a depth of 20 cm (shown in a photograph). Limit swimming times, defined as the time from the start of swimming until the mice were exhausted and sank below the surface of the water for 7 sec, were measured. The swim was performed twice, with a 60 min interval between the tests. Blood was collected 90 min after the end of the second swim, and serum QUIN and TNF- $\alpha$  levels were measured using GC/MS and the Mouse TNF- $\alpha$  ELISA Kit, respectively. For the purpose of comparison, blood was collected from age-matched non-swimming mice during the same hour. Data are represented as mean ± S.E. of 8 mice. \*p < 0.05, significantly different from non-swimming group (Student's *t*-test).

1 week of acclimation and were housed in an airconditioned room  $(23 \pm 2^{\circ}C, 55 \pm 10\%$  humidity) under a 12 hr dark/12 hr light cycle (lights on from 8:00 until 20:00), with unrestricted access to water and food. This study was approved by the Animal Experiment Committee of Kampo Research Laboratories, Kracie Pharma, Ltd. (Takaoka, Japan), in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

Weight-loaded Swimming Test —— Steel wires that weighed 5% of body weight plus 0.4 g were attached to the tails of mice with vinyl tape. Mice were forced to swim in a cylindrical acrylic tank (20 cm diameter) filled with 23°C water to a depth Under these conditions, if the mice of 20 cm. stopped swimming, they would sink into the water (shown by a photograph in Fig. 1). Limit swimming times, defined as the time from the start of swimming until the mice were exhausted and sank below the surface of the water for 7 sec, were measured. After the measurement of the limit swimming time was completed, the mice were immediately rescued from the water to avoid drowning. The swim was performed twice, with a 60 min interval between tests. Blood was collected under ether anesthesia from the orbital plexus 90 min after the end of the second swim, and the serum QUIN and tumor necrosis factor (TNF)- $\alpha$  levels were measured using the method described below. For the purpose of comparison, blood was collected from age-matched non-swimming mice during the same hour.

**Treadmill-Running Test** — Mice were first placed on the treadmill (BS-175CM; BrainScience Idea Co., Ltd., Osaka, Japan) for 30 min without

running to adapt them to the new environment. Mice were then forced to run for 60 min at 12 m/min on a 10-degree uphill incline and subsequently forced to run for 90 min with a speed increase of 2 m/min every 15 min; the final speed was 24 m/min and the total running distance was 2430 m. Blood was collected under ether anesthesia from the orbital plexus just after the end of treadmill running. Thirty minutes after blood collection, the mice were placed individually in a running wheelequipped home cage (shown by a photograph in Fig. 2; wheel diameter is 20 cm and cage size is 22  $\times$  9  $\times$  8 cm; TK-48B; Toyo-riko Co., Ltd., Tokyo, Japan). Subsequently, the wheel-running count (indicated by the counter, with 1 count corresponding to 1 revolution) was measured for 15 hr (18:00 until 9:00) under conditions that allowed the mice to take food and water and to rest freely. As we previously suggested, the wheel-running activity reflects mental activity if a running wheel-equipped home cage is used.<sup>1)</sup> The serum QUIN and TNF- $\alpha$  levels were measured using the method described below. For the purpose of comparison, blood was collected from age-matched non-running mice during the same hour, and the subsequent wheel-running count was measured in the same manner as described above.

Measurement of Serum QUIN and TNF- $\alpha$  Levels — Serum QUIN level was measured according to the method of Yu *et al.*<sup>6)</sup> using GC/MS (GC/MS-QP2010; Shimadzu Corporation, Kyoto, Japan) after the serum was appropriately diluted with pure water. The serum TNF- $\alpha$  level was measured using the Mouse TNF- $\alpha$  ELISA Kit (Thermo Fisher Scientific Inc., Rockford, IL, U.S.A.).





Mice were forced to run for 150 min on a 10-degree uphill incline (running distance was 2430 m). Thirty minutes after blood collection (described in Fig. 3), mice were placed individually in a running wheel-equipped home cage (shown in a photograph), and wheel-running count was measured for 15 hr. For the purpose of comparison, the wheel-running counts of age-matched non-running mice were measured in the same manner. Data are represented as mean  $\pm$  S.E. of 13 mice. \**p* < 0.05, significantly different from non-running group (Student's *t*-test).



**Fig. 3.** Influence of Treadmill Running on the Blood QUIN and TNF- $\alpha$  Levels in Mice

Mice were forced to run for 150 min on a 10-degree uphill incline (running distance was 2430 m). Blood was collected just after the end of treadmill running, and serum QUIN and TNF- $\alpha$  levels were measured using GC/MS and the Mouse TNF- $\alpha$  ELISA Kit, respectively. For the purpose of comparison, blood was collected from agematched non-running mice during the same hour. Data are represented as mean ± S.E. of 13 mice. \*\*\* p < 0.001, significantly different from non-running group (Student's *t*-test).

**Statistical Analysis** — Results were expressed as mean  $\pm$  S.E. Statistical differences were assessed using the Student's *t*-test. StatView Version 5.0 software (SAS Institute Japan Ltd., Tokyo, Japan) was used for the analysis, and differences were judged significant when the *p* value was less than 0.05 (*p* < 0.05).

## RESULTS

#### Weight-loaded Swimming Test

The limit swimming times in the first and the second swimming tests were  $102 \pm 17$  and  $64 \pm 14$  sec, respectively (n = 8). The serum QUIN and TNF- $\alpha$  levels of swimming mice were measured 90 min after the end of the second swim. They were about 2.5 and 1.6 times higher than those of non-swimming mice, respectively; the serum QUIN levels of swimming mice were significantly higher than those of non-swimming mice (Fig. 1).

#### **Treadmill-Running Test**

The serum levels of both QUIN and TNF- $\alpha$  were about 5 times higher in treadmill-running mice than in non-running mice, a significant increase (Fig. 3). The wheel-running counts of treadmill-running mice were measured for 15 hr after the end of treadmill running. They were significantly lower than those of non-running mice as the counts of treadmill-running mice decreased by half compared with non-running mice (Fig. 2).

#### DISCUSSION

In the weight-loaded swimming test, the serum QUIN levels of swimming mice were significantly higher than those of non-swimming mice. Similarly, in the treadmill-running test, the serum QUIN levels of treadmill-running mice were significantly higher than those of non-running mice. These results clearly indicate that blood QUIN levels are increased by exercise. Ito *et al.* reported that the blood level of kynurenine, the other TRP metabolite that precedes QUIN in the kynurenine pathway, was elevated after treadmill running in rats.<sup>7)</sup> They also showed in the same study that the activity of indoleamine 2,3-dioxygenase (IDO), the first en-

zyme in the kynurenine pathway, was increased in macrophages after treadmill running.<sup>7)</sup> These data suggest that, in the present study, TRP metabolism in the kynurenine pathway is enhanced by exercise, thereby elevating the blood QUIN level.

The serum TNF- $\alpha$  levels of treadmill-running mice were significantly higher than those of nonrunning mice. The serum TNF- $\alpha$  levels were also increased in swimming mice compared with nonswimming mice, although this effect was not significant. It has been reported that the expression of TNF- $\alpha$  mRNA in the lung is increased after treadmill running in mice.<sup>8)</sup> Also, it has been reported that blood TNF- $\alpha$  levels are elevated after a human athlete runs a marathon race.<sup>9)</sup> These reports support the results of the present study. Although TNF- $\alpha$  is reportedly produced by damage of muscle tissue,<sup>10, 11)</sup> the exact mechanism underlying the increase in TNF- $\alpha$  after exercise remains unclear. TNF- $\alpha$  is known to induce IDO activity.<sup>12)</sup> Therefore, we hypothesize that QUIN is increased after exercise through induction of IDO by TNF- $\alpha$ . Further studies are required in the future to confirm that the activity of IDO is actually increased by exercise tested in this study.

In the weight-loaded swimming test, mice were forced to swim twice, but were taken a 60 minrest between the tests. The swimming times in the first and the second swimming tests were about 100 and 60 sec, respectively. As a result, the serum OUIN and TNF- $\alpha$  levels of swimming mice were increased to about 2.5 and 1.6 times of those of nonswimming mice, respectively. On the other hand, in the treadmill-running test, mice were forced to run for 150 min on a 10-degree uphill incline without taking a rest. As a result, the serum QUIN and TNF- $\alpha$  levels of treadmill-running mice were both increased to about 5 times of those of non-running mice. These results suggest that blood levels of both QUIN and TNF- $\alpha$  increase dependently on intensity and duration of exercise.

The wheel-running counts of treadmill-running mice were significantly lower than those of non-running mice. We previously suggested that the wheel-running activity reflects mental activity of mice if a running wheel-equipped home cage is used.<sup>1)</sup> We also showed that LPS increased blood and brain QUIN levels and concomitantly decreased wheel-running activity in mice.<sup>1)</sup> Therefore, in the present study, it may be that QUIN is increased in the blood after exercise, and mental activity is thereby decreased, as in the case of LPS treatment.

IDO induction and subsequent QUIN production occur predominantly in peripheral extrahepatic organs.<sup>13, 14)</sup> QUIN does not usually enter the brain because it cannot easily pass through the bloodbrain barrier (BBB).<sup>15, 16)</sup> However, it has been reported that exercise increases the permeability of the BBB.<sup>17)</sup> Moreover, TNF- $\alpha$  is known to weaken the BBB.<sup>18, 19)</sup> Taken together, these data suggest that QUIN is produced in peripheral organs by exercise, causing increased blood QUIN levels; QUIN may then enter the brain through the weakened BBB. To confirm this hypothesis, the QUIN levels in cerebrospinal fluid (CSF) must be measured after exercise, although it is difficult to collect CSF samples from mice.

We previously showed that the number of hippocampal neurons was decreased in LPS-treated, fatigued mice.<sup>1)</sup> On the other hand, memantine, an NMDA receptor antagonist, counteracted not only the decrease in wheel-running activity but also the neuronal cell loss induced by LPS without affecting the increase in blood and brain QUIN levels.<sup>1)</sup> These findings suggest that increased brain QUIN and subsequent neuronal damage through the activation of NMDA receptors decrease mental activity in LPS-treated mice. Therefore, further studies (e.g., memantine treatment or histological observation of brain neurons) will be required in the future to clarify whether, after exercise, OUIN activates NMDA receptors and finally causes the sensation of fatigue by damaging brain neurons.

In conclusion, the present results suggest that the increase in QUIN participates in causing the sensation of fatigue after exercise. Such increase in QUIN presumably results from enhancement of TRP metabolism in the kynurenine pathway through the induction of IDO by TNF- $\alpha$ .

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