Endurance exercise increases skeletal muscle kynurenine aminotransferases and plasma kynurenic acid in humans

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Depression is a disabling mental disorder with a large impact on quality of life (9, 12). Furthermore, with almost 300 million people affected worldwide, depressive disorders are a major burden for the health care system (8, 9). Evidence-based treatment strategies include antidepressant pharmacotherapy and psychotherapy (15). Moreover, physical exercise is increasingly used to complement or substitute traditional methods even though the underlying mechanisms are not fully understood (4, 13).

A recent study showed that modifications of the kynurenine pathway are involved in the beneficial effects of exercise on depression (1). Kynurenine (KYN) is a product of tryptophan metabolism and its degradation follows one of two possible pathways: KYN is either converted to 3-hydroxykynurenine (3HK) and quinolinic acid (QUIN), a neurotoxic N-methyl-D-aspartate (NMDA) receptor agonist or to the neuroprotective NMDA receptor antagonist kynurenic acid (KYNA). In the brain, the conversion to KYNA is catalyzed by kynurenine aminotransferases (KATs) found in astrocytes (17, 18, 20). The same enzymes have been detected in peripheral tissues such as skeletal muscle (1). The relative balance between the two branches of the pathway may play an important role in the development of depressive disorders. In fact, induction of the neurotoxic branch with increased levels of cerebrospinal fluid QUIN and an increased serum QUIN-to-KYNA ratio (QUIN/KYNA) have been associated with depressive behavior (2, 5, 18, 20, 21).

Exercise activates the PGC-1α-PPARα/δ pathway which has been shown to induce skeletal muscle KAT expression (1, 19). Experiments with PGC-1α skeletal muscle-specific transgenic mice showed that an increased expression of skeletal muscle KATs shifts the KYN metabolism towards enhanced synthesis of KYNA which reduces accumulation of KYN, hereby protecting from stress-induced depression (1). Sedentary human subjects had increased skeletal muscle KAT gene expression after participating in a 3-wk endurance exercise program (1). Little is known, however, of the effect of exercise on skeletal muscle KAT protein levels and on plasma kynurenine metabolites in humans.

In the present study, we therefore compared KAT protein expression of endurance-trained and untrained subjects. In addition, we measured plasma KYN and QUIN concentrations after different types of exercise.

Materials and Methods

Human experiments. Skeletal muscle biopsies from two groups of subjects were used for analysis of mRNA and protein expression. The first group consisted of nine recreationally active male subjects who were pursuing endurance training for a 150-km road cycling time trial (END; 11.8 ± 4.8 training hours per week). The subjects of the second group (CTRL; n = 8; all male) were also recreationally active but did not specifically participate in endurance training. Skeletal muscle biopsies were taken from m. vastus lateralis under local anesthesia using the Pajunk DeltaCut system (Pajunk, Geisingen, Germany). Approximately 30 mg of muscle tissue was collected for each subject, snap frozen in liquid nitrogen, and stored at −80°C until further analysis. Local compression was applied on the biopsy site for a few hours.

Blood plasma was collected from both groups at rest and from the END group several time points after completion of the 150-km road cycling time trial. In addition, we collected plasma from another group of recreationally active subjects (n = 11; 10 male, 1 female) before and after running a half-marathon race on a hilly course. Furthermore, plasma was collected from a third group of subjects before and after performing eccentric exercise that consisted of a series of 100 drop jumps from a 50-cm platform with active deceleration to 90° knee angle, immediately followed by a maximal rebound jump (n = 9; all male). Venous blood samples were collected in

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EDTA-treated vacutainers and centrifuged for 15 min (3,000 rpm and 4°C). Plasma was then transferred to Eppendorf tubes and stored at −80°C until analysis. The study was approved by the local ethics committee and each subject provided written informed consent before participation.

Western blot analysis. Frozen human vastus lateralis biopsies were homogenized in ice-cold homogenization buffer (20 μl/mg muscle) with a ground glass homogenizer. The buffer (pH 7.6) consisted of 20 mM HEPES, 150 mM NaCl, 5 mM EDTA, 25 mM NaF, 1 mM Na3VO4, 20% glycerol (vol/vol), 0.5% Triton X-100 (vol/vol), and 1 tablet per 50 ml protease inhibitor cocktail (no. 11836145001, Roche).

Homogenates were rotated for 30 min at 4°C and subsequently centrifuged at 700 g and 4°C for 10 min. Protein content of the supernatant was quantified using the Bradford assay (no. 500-0006, Bio-Rad) and samples were diluted 1:1 in Laemmli sample buffer (no. 161-0737EDU, Bio-Rad) with 5% β-mercaptoethanol (vol/vol) and heated at 95°C for 5 min.

Protein (10 μg/well) was separated on a 4–12% precast Bis-Tris gel (NP0336PK2, NuPAGE, InVitrogen) and transferred to polyvinylidene fluoride membranes (Immobilon, Millipore, Billerica, MA). Membranes were blocked at room temperature for 1 h with LI-COR blocking buffer (927-40000, LI-COR) and incubated overnight at 4°C with the following antibodies diluted in blocking buffer: 1:1,000 rabbit anti-KAT1 (anti-CCB1, 12156-1-AP, Proteintech, Manchester, UK), 1:1,000 rabbit anti-KAT2 (anti-KATII/AADAT, LS-C309277/65009, LifeSpan Biosciences, Seattle, WA), 1:200 rabbit anti-KAT3 (anti-CCB2L, HPA026538, Atlas Antibodies, Stockholm, Sweden), 1:800 rabbit anti-KAT4 (Antibodies-Online, Aachen, Germany), and mouse anti-actin (ab3280, Abcam, Cambridge, UK). While the antibodies for KAT 1, KAT 3, and KAT 4 clearly gave one specific band, the KAT 2 antibody showed more than one band per lane and only the band at the specific size of KAT 2 (47 kDa) was quantified. After washing with Tris-buffered saline-Tween 20, membranes were incubated with secondary antibody [1:15,000 IRDye 680-conjugated donkey anti-mouse IgG and IRDye 800-conjugated donkey anti-rabbit IgG in blocking buffer (926-680792 and 926-32213, LI-COR)] at room temperature for 1 h.

Bands were visualized by infrared fluorescence (IR-Odyssey scanner, LI-COR Biosciences) and band densities were quantified using LI-COR software, LI-COR (version 2.0.38; LI-COR Biosciences) software. All values were normalized to β-actin.

Analysis of gene expression. Isol-RNA Lysis Reagent (5 PRIME) was used to isolate total RNA from the muscle biopsies. Amplification Grade DNase 1 (Life Technologies) was added to 1 μg of RNA and from that, 500 ng were used for cDNA preparation with the Applied Biosystem Reverse Transcription Kit (Life Technologies). A ViiA 7 Real-Time PCR system thermal cycler with SYBR Green PCR Master Mix (both Applied Biosystems) was used for quantitative real-time PCR. Gene expression analysis was performed using the ΔΔCt method and all values were normalized to hypoxanthine phosphoribosyltransferase (HPRT) mRNA levels relative to controls.

High-performance liquid chromatography. The plasma samples were diluted (1:2, vol/vol) with 0.4 M perchloric acid (PCA) containing 0.1% sodium metabisulfite and 0.05% EDTA and then centrifuged for 5 min at 21,000 g immediately before analysis. The supernatant was further diluted (1:1,10, vol/vol) with 70% strength PCA and re-centrifuged for 5 min at 21,000 g. The supernatant was transferred to a new Eppendorf tube for high-performance liquid chromatography (HPLC). HPLC was performed using an isocratic HPLC system with a dual-piston, high-pressure delivery pump (LC 10AD Shimadzu, Japan) and a ReproSil-Pur C18 column (4 × 150 mm, Dr. Maisch, Ammerbuch, Germany). A fluorescence detector (Jasco FP-2020, Hachioji City, Japan) with an excitation wavelength of 344 nm and an emission wavelength of 398 nm (18 nm bandwidth) was used for detection.

A mobile phase (7.0% acetonitrile and 50 mM sodium acetate in ultrapure dH2O; pH 6.20 adjusted with acetic acid) was pumped through the column at a flow rate of 0.5 ml/min. Twenty-microliter samples were manually injected into a Rhodineyve injector (Rhonet Park, CA) with a 100-μl single sample loop volume. A zinc acetate (0.5 M not pH adjusted) buffer was added postcolumnar (pump P-500 Pharmacia, Uppsala, Sweden) at a flow rate of 10 ml/h. The fluorescence signals were digitized and analyzed with Datalys Azur Software (Grenoble, France). The concentration of KYNA was extrapolated from daily prepared standard curves by dilution of aliquots. The retention time for KYNA was 7.8 min. The limits of detections were at least 20 times lower than the reported plasma concentrations.

QUIN analysis was performed with liquid chromatography/tandem mass spectrometry at BEVITAL AS (Norway) as previously described (16).

Statistical analysis. Statistical analyses were performed using GraphPad Prism 6. Data are presented as means ± SE, and P ≤ 0.05 was considered to be significant. Differences in KYNA plasma concentrations and QUIN/KYNA ratio before and after exercise were assessed with one-way repeated-measures ANOVA followed by a Tukey post hoc test. Unpaired t-tests were used to compare gene and protein expression between different groups. Correlations were calculated by Spearman rank correlation.

RESULTS

Gene and protein expression in skeletal muscle. To investigate whether practicing endurance exercise on a regular basis can affect skeletal muscle KAT expression, we compared muscle biopsies from actively endurance trained (END) and control subjects (CTRL). Changes in the expression of KAT genes and its upstream regulatory pathway PGC1α1-PPARsβ/δ were analyzed. Muscle biopsies from the END subjects displayed higher mRNA expression for KAT isoforms 1–4 than the CTRL group (Fig. 1A). Moreover, mRNA expression of PGC1-α1 and PPARα genes were significantly higher in the END subjects than in CTRL, whereas PPARδ was similar in the two groups (Fig. 1A). To further confirm that the PGC1-α1 pathway was activated in the END group, the PGC1-α1 downstream genes CPT1B, VEGFA, and PDK4 were analyzed and all three showed higher expression in END than in CTRL (Fig. 1A).

The expression of PGC1-α1 and PPARα genes directly correlated with KAT 4 gene expression (Fig. 1B).

To further confirm that the observed changes in KAT gene expression correspond to changes in protein levels, we performed immunoblotting using specific antibodies. KAT protein expression was significantly higher in the END group than in the CTRL group (Fig. 2, A and B).

Plasma kynurenine metabolites. To study changes in kynurenine metabolism during exercise, we measured KYNA and QUIN concentrations and calculated the QUIN/KYNA ratio in plasma samples from subjects before and after performing different types of exercise.

One hour after a 150-km road cycling time trial, plasma KYNA was substantially increased by 63% (Fig. 3A), whereas QUIN only increased by 19% (338 ± 16 mM to 404 ± 30 mM; n = 9, P < 0.05). The QUIN/KYNA ratio decreased after exercise by 27% (Fig. 3B). The KYNA concentration and QUIN/KYNA ratio returned to baseline within 24 h (Fig. 3, A and B). To further confirm that endurance exercise leads to increased plasma KYNA, another group of subjects was analyzed before and after performing a half- marathon race on a hilly course. This group also showed an increase (125%) in plasma KYNA 30 min after finishing the race (36 ± 3 mM to 0.05)
endurance exercise causes a transient increase in plasma KYNA concentration and a reduction in the QUIN/KYNA ratio. Moreover, in endurance-trained individuals, long-term muscle adaptations in the KYN metabolism occur by increased expression of KAT genes and proteins, which is linked to upregulation of PGC-1α and PPARα gene expression.

Recreationally active humans who were engaged in endurance exercise on a regular basis had higher skeletal muscle expression of KAT genes and proteins, which is linked to muscle adaptations in the KYN metabolism occur by increased ratio. Moreover, in endurance-trained individuals, long-term muscle adaptations in the KYN metabolism occur by increased expression of KAT genes and proteins, which is linked to upregulation of PGC-1α and PPARα gene expression.

Recirculation active humans who were engaged in endurance exercise on a regular basis had higher skeletal muscle expression of KAT genes and proteins, which is linked to muscle adaptations in the KYN metabolism occur by increased expression of KAT genes and proteins, which is linked to upregulation of PGC-1α and PPARα gene expression.

**DISCUSSION**

In the present study, we investigated whether regular participation in endurance exercise can lead to long-term adaptations of peripheral KYN metabolism in humans. We show that

81 ± 8 nM, n = 11, P < 0.0001). In contrast, plasma concentration of KYNA, QUIN, and the QUIN/KYNA ratio were not changed in subjects that performed eccentric exercise consisting of a series of 100 drop jumps (Fig. 3, C and D). In summary, plasma concentration of KYNA increases and the QUIN/KYNA ratio decreases transiently following endurance exercise but not after eccentric muscle work.
KAT gene expression than subjects with no specific endurance training. These results are consistent with a previous study that reported an increase in KAT gene expression in sedentary subjects after a 3-wk endurance exercise program (1). That study also found elevated PGC-1α1, PPARα, and PPARδ gene expression after exercise and demonstrated that activation of the PGC-1α1-PPARα/δ pathway is the underlying mechanism for KAT upregulation. We also observed higher PGC-1α1 and PPARα gene expression for the END subjects, but PPARδ gene expression did not differ between the two groups. In further support for an increased activity of the PGC-1α1 pathway, we observed increased expression of the PGC-1α1 downstream genes CPT1B, VEGFA, and PDK4 in endurance athletes.

To our knowledge, only one previous study has investigated the effect of exercise on skeletal muscle KATs in humans (1). That study reported only mRNA expression which is not always mirrored by a corresponding increase in protein levels. We here show that humans participating in active endurance training display both an increased mRNA expression of the KAT genes as well as increased protein levels, which supports a mechanism of exercise-induced adaptations in the KYN pathway as previously reported (1).

We show that, following aerobic endurance exercise, a transient flux of the kynurenine pathway occurs with a preferential increase in the KYNA concentration (63%) over QUIN (19%), leading to a decreased QUIN/KYNA ratio. This would have relevance for patients with major depression as an increased serum QUIN/KYNA ratio has been linked to patients with depressive disorder. Interestingly, the QUIN/KYNA ratio was reduced when depressive patients were treated with electroconvulsive therapy (21).

To further investigate how other types of exercise affect KYN metabolism, we analyzed plasma samples of subjects who performed eccentric exercise (100 repeated drop jumps). While this type of exercise is considerably less energetically demanding than running or cycling, it still exerts substantial stress on the muscle and induces extensive muscle soreness (11). Nevertheless, the eccentric exercise protocol did not cause any change in plasma KYNA or the QUIN/KYNA ratio. A recent study that screened for plasma metabolite changes following exercise reported an increase in plasma KYNA after marathon running but not after a short treadmill exercise test (14). In summary our results suggest that the exercise-induced enhancement of the KYNA pathway is linked to endurance exercise with a prolonged and high metabolic demand rather than high force eccentric contractions.

The increase in plasma KYNA can possibly depend on differences in the availability of free tryptophan (TRP). At rest, the majority of TRP in the blood is bound to albumin and only a small proportion is available in the free form. Free fatty acids (FFA) competitively bind to albumin, and dissociate the bound TRP thus increasing the concentration of free TRP in the blood (3, 7). It is possible that the increased lipolysis and subsequent rise in plasma FFA, which occurs during prolonged endurance exercise (10), causes an increase in free TRP and thus an increased flux through the kynurenine pathway.

In our study, resting plasma KYNA was similar in all subjects. After endurance exercise, plasma KYNA concentration increased transiently. In this context it is noteworthy that circulating KYNA is rapidly excreted by the kidneys, which likely contributes to the decay of KYNA following exercise (6). Thus transient and repeated, rather than sustained, increases in plasma KYNA would be the link between KYNA-induced reduction of depressive symptoms and exercise in humans. This notion can have bearing on exercise recommendations for patients with depression, which then should be focused on repeated endurance-type training.

Our results show that recreationally active humans who engage in regular endurance training have increased skeletal muscle KAT gene and protein expression. Mice with increased muscular KAT levels have been shown to be resilient to stress-induced depression due to an enhanced peripheral conversion of KYNA to KYNA (1). Our study supports a similar mechanism also in humans. This highlights the effect of exercise on KYNA metabolism in humans and opens up for further mechanistic understanding on exercise as a treatment for depressive disorders.


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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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9. Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301


