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Research article

Do Acute Exercise-Induced Activations of the Kynurenine Pathway Induce Regulatory T-Cells on the Long-Term? – A Theoretical Frame Work Supported by Pilot Data

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Abstract

Regular physical activity and exercise interventions are suspected to have anti-inflammatory effects depending on exercise modality, thereby potentially reducing the risk and progress of several chronic diseases. Alterations in the kynurenine pathway may represent a link between inflammatory responses following acute exercise and chronic anti-inflammatory properties, such as increased levels of regulatory T-cells (T_{reg}). Here, we hypothesize that acute exercise activates the kynurenine pathway and physical fitness is associated with proportions of circulating anti-inflammatory T_{reg} in older healthy women. Nineteen older healthy female participants (55 years (SD: ± 5.6)) completed a cardiopulmonary incremental exercise test (CPET) with spirometry on a bicycle ergometer until exhaustion with maximum oxygen uptake (VO_2max) as outcome. Blood samples were taken before (T0) and one minute after (T1) the CPET. Levels of tryptophan, serotonin and kynurenine were determined by enzyme-linked immunosorbent assays. Flow cytometry was used to identify proportions of T-cell subsets. Both, kynurenine ($p = 0.003$, $d = 0.40$) and the kynurenine/tryptophan ratio ($p = 0.034$, $d = 0.48$) increased significantly after acute exercise. Moreover, participants` VO_2max was strongly correlated with T_{reg} levels ($p < 0.001$, $r = 0.689$). This is the first study indicating a kynurenine pathway activation following acute exercise in older healthy women. The observed correlation between T_{reg} levels and VO_2max emphasizes a potential link between short-term upregulated kynurenine levels and longer-term anti-inflammatory properties of exercise. Future research is needed to clarify to what extend acute exercise-induced activations of the kynurenine pathway contribute to T_{reg} differentiation.

Key words: Acute exercise, kynurenine pathway, immune cells, regulatory T-cells, tryptophan.

Introduction

Regular physical activity and exercise are associated with a reduced risk and delayed progress for several chronic cardio-vascular (Lavie et al., 2015) and neurodegenerative diseases (Cass, 2017; Grazina and Massano, 2013) and cancer (Kerr et al., 2017; Cormie et al., 2017). The knowledge about the underlying mechanisms is still sparse. However, a vast body of literature suggests that these positive effects of exercise are at least partially driven by its long-term anti-inflammatory properties (Gleeson et al., 2011). Thereby, exercise may reduce a common risk factor for all diseases mentioned above (Gleeson et al., 2011; Nathan, 2002; Walsh et al., 2011).

So far, two general mechanisms have been linked to the long-term anti-inflammatory potential of exercise. First, exercise can help to reduce visceral fat mass, a major source of inflammatory factors. Second, regular exercise is known to increase the body`s anti-inflammatory potential (Gleeson et al., 2011). Recently, we have shown that endurance capacity in a large cohort of top athletes is positively correlated with numbers and proportions of circulating regulatory T-cells (T_{reg}) (Weinhold et al., 2016), representing main producers of anti-inflammatory cytokines, e. g. Interleukin 10 and TGF-ß. Moreover, athletes indicated elevated circulating T_{reg} numbers and proportions compared to age matched controls. In detail, it was hypothesized that the short-term pro-inflammatory stimulus which is well known to be induced by each bout of physical exercise provokes a long-term increase/adaption in the body`s anti-inflammatory potential. A mechanistic model for this adaption is still missing.

A potential explanation may be derived from research in the oncological setting. A number of studies have shown that tumor cells indicate an elevated activation of tryptophan (TRP) breakdown through the kynurenine (KYN) pathway (Platten et al., 2014). More detailed, inflammatory signaling induces the indoleamine-2,3-dioxygenase 1 (IDO1), the initial enzyme of the KYN pathway which metabolizes TRP to KYN. KYN itself has several immune-modulating properties. So far, it was shown that KYN induces T_{reg} differentiation (Platten et al., 2014; Chung et al., 2009). Interestingly, first studies have shown that an acute bout of exercise strongly activates the KYN pathway on the short-term (reviewed by (Metcalfe et al., 2018)).

Here, we investigate whether i) an acute bout of endurance exercise activates the kynurenine pathway and ii) physical fitness is positively associated with resting levels

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of anti-inflammatory T_{reg} in a population at risk for all diseases mentioned above (older women).

Methods

This study was performed in accordance with the declaration of Helsinki, under consideration of the standards for ethics in sport and exercise research and approved by the ethics committee of the University Hospital of Cologne.

Inclusion criteria

The following in- and exclusion criteria were defined: healthy female subjects in a state of complete physical, mental, and social well-being as defined from World Health Organization (WHO), age above 50 years, no chronic medication intake, no chronic internal disease, no orthopaedic problems, no physical impairments and any other concomitant malignant diseases that would rule out participation in the cardiopulmonary exercise testing (CPET).

Experimental design

Subjects were recruited by announcement in the German Sport University of Cologne website. All participants provided written consent to participate before the beginning of the experiment. To determine acute effects of endurance exercise on the KYN pathway, venous blood samples were collected before (T0) and one minute after (T1) a CPET (described below) by a venipuncture.

Cardiopulmonary exercise testing (CPET)

The CPET was performed on a bicycle ergometer (ergoline GmbH, Bitz, Germany). The measurement protocol (Cortex Biophysik GmbH, Leipzig, Germany) started with an one minute rest measurement, followed by a three minute warm-up phase at 50 watts of power output and an increase of 25 watts every two minutes until exhaustion of the subject (respiratory exchange ratio>1). In parallel, heart rate recordings were performed through an electrocardiogram (Promedia Medizintechnik, Siegen, Germany) during the whole testing.

Peripheral blood mononuclear cells isolation (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation with lymphocyte separation medium (Promo Cell, Heidelberg, Germany) for 30 minutes at 800g. Subsequently, PBMCs were washed with PBS and resuspended in freezing medium (Thermo Fischer, Waltham, USA) for storage at -150°C until measurement.

Flow cytometry (FACS)

After thawing, isolated PBMCs were stained with antibodies for CD3, CD4, CD8, CD25 and CD127. (BD Bioscience, Heidelberg, Germany). T-cells were gated as $CD3^+$ lymphocytes and further divided into T-helper cells (T_{helper}) ($CD4^+$) and $T_{cytotox}$ ($CD8^+$). Proportions of T_{reg} were gated as $CD25^+$ $CD127^{dim}$ cells of the T_{helper} population. Flow cytometry was conducted on a BD FACS Array (BD Bioscience, Heidelberg, Germany).

Enzyme linked Immunosorbent Assay (ELISA)

Plasma total TRP, KYN and SER levels were analyzed for both time points by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (IDK® Tryptophan, IDK® Kynurenine and IDK® Serotonin, Immundiagnostik GmbH, Bensheim, Germany). KYN/TRP ratio was calculated to indirectly assess TRP breakdown within the KYN pathway.

Statistical analysis

All statistical analyses were conducted using SPSS software, version 24.0 (IBM, Armonk, New York). Data are presented with means and standard deviation. Normal distribution of all variables was first assessed by Kolmogorov-Smirnow-Test. Not normally distributed variables were logarithmically corrected (KYN, TRP, SER, KYN/SER, TRP/SER), and re-tested for normality. All variables met the normal distribution after correction. Multiple t-tests were used to compare the blood markers levels pre and post exercise. Cohen's d effect sizes were reported. Pearson correlations were then used to evaluate the association between markers. Level of significance for all statistical analyses was set at alpha = 5%.

Results

The 19 recruited healthy women had a mean age of 55 years (SD: ± 5.6), a mean BMI of 22.3 $kg \cdot m^{-2}$ (SD: ± 2.6) and a mean VO_2max of 33.3 $ml \cdot min^{-1} \cdot kg^{-1}$ (SD: 6.2), mean power output 181.6 W (SD: ± 26.1), mean duration 15.8 minutes (SD: 1.9), mean respiratory quotient 1.22 (SD: 0.11), mean maximum heart rate 171 bpm (SD: 12).

Mean values and SDs of all outcome measures are shown in Figure 1. Our results indicate a significant increase of participants' KYN ($p = 0.003$, $d = 0.40$) and KYN/TRP ratio ($p=.034$, $d=.48$) after the endurance exercise. The markers of the serotonergic pathway (SER levels, TRP/SER, KYN/SER) as well as the plasma total TRP were not significantly affected by exercise.

Correlational analyses are presented in Table 1. Participants` VO_2max strongly positively correlated with T_{reg} levels ($r = 0.689$; $p < 0.001$, $n =19$). In contrast, TRP levels were inversely associated with participants' VO_2max ($r = -0.601$; $p < 0.01$, $n = 19$). Additionally, T_{reg} levels were strongly correlated with $T_{cytotox}$ ($r = 0.673$; $p <0 .005$, $n =19$).

Discussion

To our knowledge, it is reported for the first time that acute exhaustive endurance exercise significantly activates the kynurenine pathway in older healthy female subjects. Moreover, it was shown that endurance capacity, as an indirect marker of training status, is positively associated with T_{reg} levels in this population.

Previous studies have shown that a single bout of exhaustive endurance exercise is able to activate the kynurenine pathway (Metcalfe et al., 2018), a fact that is in accordance to the increased kynurenine and KYN/TRP ratio of the sample examined. The observed activation of the kynurenine pathway may be mediated through a short-term

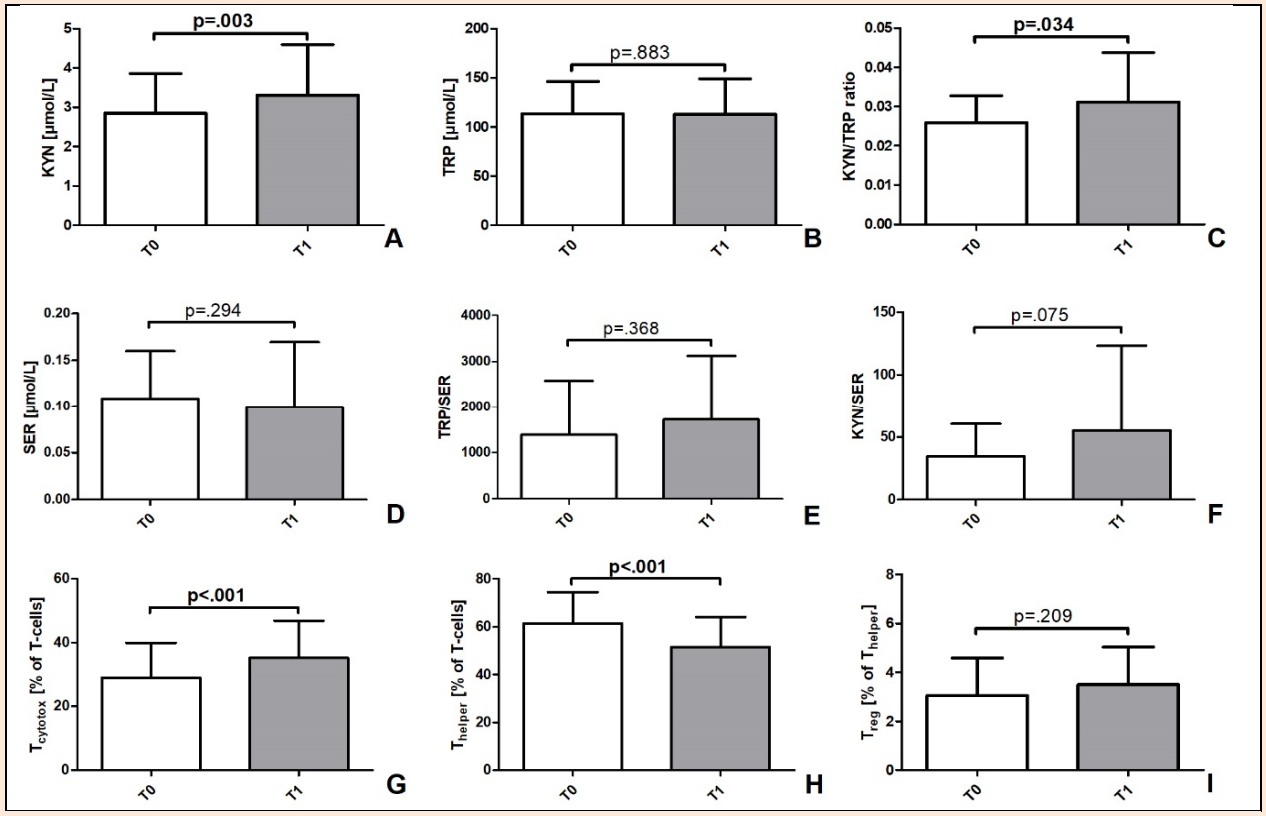

Figure 1. Bar graphics of participants' blood markers (N=19) at T0 and T1. Values are presented as mean and standard deviation.

Table 1. Pearson correlations between participants' blood markers (n = 19) at baseline.

	BMI	TRP	KYN	KYN/TRP	Tcytotox	Treg
VO_2max	-.659**	-.601**	-.278	.454	.287	.689***
TRP			.716***	-.427	.017	-.293
KYN				.325	.252	-.109
KYN/TRP					.304	.257
Tcytotox						.673***

*** p<0.001; ** p<0.01; BMI: Body Mass Index; KYN: kynurenine; TRP: tryptophan; $T_{cytotox}$: cytotoxic T cells; T_{reg}: regulatory T cells. VO_2max: maximal oxygen uptake.

inflammatory signal (Wang et al., 2015), as it is provoked by acute exercise. In detail acute exercise elevates serum levels of pro-inflammatory cytokines, such as Interleukin-1 or Interleukin-6 (Walsh et al., 2011, (Pedersen, 2009; Fischer, 2006). Nevertheless, acute exercise is also suspected to alter blood cortisol and free tryptophan concentrations (O`Connor and Corrigan, 1987; Strasser et al. 2016). Both factors have previously been described as activators of the tryptophan 2,3 dioxygenase (TDO), a consecutive expressed liver-specific isoenzyme of IDO (Badawy, 2017). However, TRP levels did not change significantly in our study. In contrast Strasser et al. reported a significant decrease of TRP after exercise. It is worth mentioning that both studies are hardly comparable due to differences in participants (older women vs. young athletes) and exercise protocols/duration (CPET: 15.8 minutes vs. CPET + 20 minutes maximum time trial >30 minutes overall). Regarding cortisol, studies reveal inconsistent results on acute exercise-induced effects (Hayes et al. 2015). Therefore, it cannot be ruled out that the observed increase in kynurenine and KYN/TRP ratio is also driven by an induction of TDO.

In contrast to the increased KYN/TRP ratio, results of this study show no changes on the TRP/SER ratio. This finding emphasizes that acute exercise predominately activates the kynurenine pathway, without impairing peripheral serotonin metabolism.

We have previously revealed that enhanced endurance capacity is associated with higher T_{reg} numbers and proportions in athletes (Weinhold et al., 2016). In line with these results, a similar association was found in the present investigation. Therefore, the results of this study cautiously support the initial hypothesis that repeated short-term increases of kynurenine levels, as they appear after each bout of exercise may lead to a long-term increase in T_{reg} (see Table 1). We are aware that more research is needed to improve this hypothesis on a mechanistic level as demonstrated in Figure 2. Nevertheless, it should be kept in mind that the described link between acute increases in biomarkers (here kynurenine) and long-term adaptions (here

induction of T_{reg}) is generally hard to prove in longitudinal in vivo studies.

The results of the present investigation should be read within the context of its strengths and limitations. Limitations include a relatively small sample size, lack of control group, lack of subjects' physical activity/ training behavior documentationlack of follow up measurements,and lack of gene expression or protein level analyses. Further research may include different clinical populations, a wider range of markers such as kynurenic acid and quinolinic acid to cover the neuroregulatory bench of the KYN pathway. Additionally, potential mediators, which are known to be involved in KYN pathway regulation, such as cortisol and inflammatory factors (e. g. Interleukin-6) should be investigated. Strengths of this study include the involvement of a homogenous population at risk for diseases as well as an approach combining the effect of exercise on the KYN pathway and its relation to immune system markers.

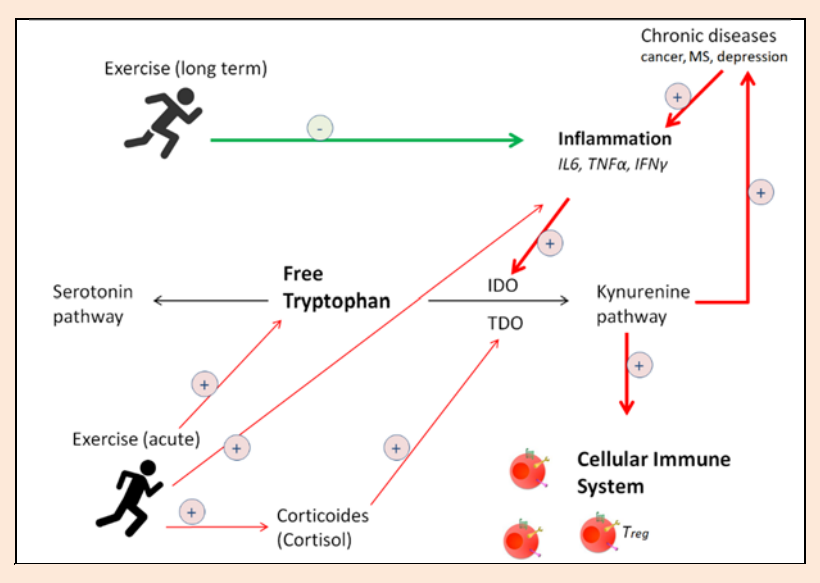

Figure 2. Influence of exercise and inflammation on the kynurenine pathway. Thin colored arrows represent an acute effect and bold colored arrows represent a long-term effect. IDO: Indoleamine-2,3-Dioxygenase; IFN: interferon [IFN]-γ; IL6: interleukin 6; TDO: Tryptophan-2,3-Dioxygenase; TNFα: Tumor necrosis factor α; $T_{cytotox}$: cytotoxic T cells; T_{helper}: T helper cells; T_{reg}: regulatory T-cells.

Conclusion

In conclusion, older women indicated an activation of the kynurenine pathway in response to a single bout of exhaustive endurance exercise. More research is warranted to clarify whether this activation is rather driven by altering IDO1 or TDO induction and activity. Additionally, previously described associations between fitness and T_{reg} proportions (as a marker of anti-inflammatory potential) in athletes was approved for older women. Further investigations will be necessary to improve our hypothesis that an increase in T_{reg} is induced by repetitive exercise induced increases in kynurenine levels.

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Key points

- The Kynurenine Pathway can be activated by acute exercise in older healthy women
- Higher VO_2max values are associated with increased levels of regulatory T-cells
- Both findings within one sample suggest a potential interaction between acute exercise-induced Kynurenine Pathway activation and chronically elevated anti-inflammatory capacity

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