Institute of Cardiovascular Research and Sport Medicine

German Sport University Cologne

Head of the Institute: Univ.-Prof. Dr. Wilhelm Bloch

Acute and chronic effects of endurance exercise on Tryptophan metabolism in middle aged women and persons with MS

Doctoral thesis accepted for the degree

PhD in Natural Sciences

by

Christina Koliamitra

from

Volos, Greece

Cologne 2021

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First Reviewer:	Univ.-Prof. Dr. Wilhelm Bloch
Second Reviewer:	JProf. Dr. Dr. Philipp Zimmer
Chair of the doctoral committee:	Univ.-Prof. Dr. Mario Thevis
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Affidavits following §7 section 2 No. 4 and 5 of the doctoral regulations from the German Sport University Cologne, February 20th 2013:

Hereby I declare:

The work presented in this thesis is the original work of the author except where acknowledged in the text. This material has not been submitted either in whole or in part for a degree at this or any other institution. Those parts or single sentences, which have been taken verbatim from other sources, are identified as citations.

I further declare that I complied with the actual “guidelines of qualified scientific work” of the German Sport University Cologne.

_____

Date, Signature

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Last but not least, I would like to thank all the patients that I worked with, examined or met during all these years and numerous studies for giving me the most valuable life lessons and making me wiser.

List of Contents

Abbreviations

Trp	Tryptophan
Kyn	Kynurenine
MS	Multiple sclerosis
T_{regs}	Regulatory T-cells
RRMS	Relapsing remitting multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
TDO	Tryptophan 2,3-dioxygenase
IDO	Indoleamine 2,3 dioxygenase
KMO	Kynurenine-3-monooxidase
AIDS	Acquired Immune Deficiency Syndrome
CNS	Central nervous system
LAT	Large amino acids transporter
KYNA	Kynurenic acid
QA	Quinolinic acid
NMDA	N-methyl-d-aspartate
nAChRs	Nicotinic acetylcholine receptors
KAT	Kynurenine aminotransferase
3-HK	3-hydroxykynurenine
3- HAA	3- hydroxyanthranilic acid
NAD	Nicotinamide adenine dinucleotide
CoA	Coenzyme A
PBMC	Peripheral blood mononuclear cell
IFN-y	Interferon Gamma
IL	Interleukin
NK	Natural Killer
AhR	Aryl Hydrocarbon Receptor
LPS	Lipopolysaccharide
TNF	Tumor Necrosis Factor
iNKT	Natural Killer T cells
GPR35	G-protein coupled receptor 35
ROS	Reactive Oxygen Species
CD	Cluster of Differentiation
RBC	Red Blood Cells
CPET	Cardiopulmonary Exercise Testing
VO_{2max}	Maximum oxygen consumption
SER	Serotonin
ELISA	Enzyme-linked immunosorbent assay
HPLC	High Performance liquid chromatography
HIT	High Intensity Training
CT	Control Training
5HT	5-hydroxytryptamine

Abstract

Tryptophan (Trp) is an essential amino acid involved in different biological processes. One essential pathway in TRP metabolism is Kynurenine (Kyn) pathway which, when at a central level comports neuro-protective and neuro-toxic metabolites. Higher breakdown of Trp to Kyn during inflammatory conditions indirectly results in an elevated Kyn/Trp ratio. Metabolic disorders of the Kyn pathway are linked to the pathogenesis and progression of various chronic diseases. Increased tryptophan utilization is also reported during autoimmune and inflammatory diseases such as Multiple Sclerosis (MS). It has been shown that acute and chronic exercise has strong influence on Trp metabolism. Systematic physical exercise has anti-inflammatory effects depending on the exercise modality, thus potentially reducing the risk and progress of several chronic diseases. Modifications in the Kyn pathway may signify a link between inflammatory responses following acute exercise and chronic anti-inflammatory properties, such as increased levels of regulatory T cells (T_{regs}), a subpopulation of T cells that modulates the immune system, sustains tolerance to self-antigens, and prevents autoimmune diseases.

In this thesis two original investigations were conducted on the effect of chronic and acute exercise on Kyn Pathway in persons with MS and healthy women. Supplementary aim of this thesis was to investigate any possible connection with T_{regs} and the Kyn Pathway influence.

In summary, this thesis demonstrates the different responses of Trp metabolism to acute and chronic exercise in persons with relapsing remitting MS (RRMS) and secondary progressive MS (SPMS). Serotonin increased after training, whereas the kynurenine pathway was only activated in persons with RRMS. The second study conducted was the first to indicate a kynurenine pathway activation following acute exercise in older healthy women. A correlation between T_{reg} levels and maximum oxygen capacity emphasizes a potential link between short-term upregulated Kyn levels and longer-term anti-inflammatory properties of exercise.

Future research is warranted to investigate if the described alterations influence targets of Trp metabolites, such as immune function and cognitive performance in persons with MS. Additional research is needed to clarify to what extend acute exercise-induced activations of the kynurenine pathway contribute to T_{reg} differentiation.

Zusammenfassung

Tryptophan (Trp) ist eine essentielle Aminosäure, die an verschiedenen biologischen Prozessen beteiligt ist. Ein wesentlicher Abbauweg des Trp Metabolismus ist der Kynureninpfad, der neuroprotektive und neurotoxische Metaboliten aufweist. Ein größerer Abbau von Trp zu Kynurenine (Kyn) unter entzündlichen Bedingungen führt indirekt zu einem erhöhten Kyn / Trp Ratio. Fehlregulationen des Kynureninpfads hängen mit der Pathogenese und dem Fortschreiten verschiedener chronischer Krankheiten zusammen. Ein erhöhter Tryptophanabbau wird auch bei Autoimmunerkrankungen und entzündlichen Erkrankungen wie Multipler Sklerose (MS) berichtet. Es wurde gezeigt, dass akutes und chronisches Training einen starken Einfluss auf den TRP Metabolismus hat. Systematische körperliche Aktivität wirkt in Abhängigkeit von der Belastungsmodalität entzündungshemmend und verringert somit möglicherweise das Risiko und den Fortschritt verschiedener chronischer Krankheiten. Modifikationen im Kynureninpfad könnten einen Zusammenhang zwischen Entzündungsreaktionen nach akuten Belastungen und chronischen entzündungshemmenden Eigenschaften bilden, wie z. B. erhöhte Spiegel an regulatorischen T-Zellen (T_{reg}), einer Subpopulation von T-Zellen, die das Immunsystem modulieren, die Toleranz gegenüber Antigene aufrechterhalten, und Autoimmunerkrankungen vorbeugen.

In dieser Arbeit wurden zwei Originalarbeiten zum Effekt chronischer und akuter körperlicher Aktivität auf den Kynureninpfad bei Personen mit MS und gesunden Frauen durchgeführt. Zusätzliches Ziel dieser Arbeit war es, einen möglichen Zusammenhang mit regulatorischen T-Zellen und die Wirkung des Kynureninpfads zu untersuchen.

Zusammenfassend konnte diese Arbeit die unterschiedlichen Reaktionen des Trp Metabolismus auf akutes und chronisches Training bei Personen mit schubförmig remittierende (RRMS) und sekundär-progrediente MS (SPMS) zeigen. Serotonin stieg nach dem Training an, während der Kynureninpfad nur bei Personen mit RRMS aktiviert wurde. Die zweite durchgeführte Studie war die erste, die auf eine Aktivierung des Kyn Pathway nach akutem Training bei älteren gesunden Frauen hinwies. Eine Korrelation zwischen den T_{regs} und der maximalen Sauerstoffkapazität unterstreicht einen möglichen Zusammenhang zwischen den kurzfristig hochregulierten Kyn Leveln und den längerfristigen entzündungshemmenden Eigenschaften des Trainings.

Zukünftige Forschung sollte untersuchen, ob die beschriebenen Veränderungen die Ziele von Trp-Metaboliten beeinflussen, wie z. B. die Immunfunktion und die kognitive Leistung bei Personen mit MS. Zusätzliche Untersuchungen sind erforderlich, um zu klären, inwieweit akute belastungsinduzierte Aktivierungen des Kynureninpfads zur T_{reg} -Differenzierung beitragen.

1. Introduction

Tryptophan (Trp) is an essential amino acid involved in different biological processes. Tryptophan contains an α-amino group, an α-carboxylic acid group, and a side chain indole, making it a non-polar aromatic amino acid. It is important in humans and since the human body cannot synthesize it, it must be obtained through nutrition in order to maintain the nitrogen balance in the human body (Slominski et al., 2002, Cervenka et al., 2017b). There are two antagonistic pathways in Trp metabolism: the serotonergic pathway, that results in the production of monoaminergic neurotransmitters (e.g. serotonin and melatonin) regulating human behavior, appetite and tiredness (Badawy, 2017; Cervenka et al., 2017; Leklem, 1971) and the kynurenine (Kyn) pathway which, when at a central level comports neuro-protective (e.g. Kyn, kynurenic acid) and neuro-toxic metabolites (e.g. quinolinic acid, 3-hydroxykynurenin). Only one percent of the essential amino acid Tryptophan is used for protein synthesis under physiological conditions. While it is just a small portion metabolized through the serotonergic pathway, the highest portion of available Trp is metabolized through the Kyn pathway by the liver-specific enzyme tryptophan 2,3-dioxygenase (TDO) (Badawy, 2017; Cervenka et al., 2017; Chen & Guillemin, 2009; Leklem, 1971). However, Trp metabolism can additionally take place in the lungs, kidneys, spleen, placenta and blood cells by the enzyme indole 2,3-dioxygenase (IDO) (Braidy et al., 2011; Chen & Guillemin, 2009; Strasser et al., 2017).

Higher breakdown of Trp to Kyn during inflammatory conditions redirects an increase in the activity of IDO, which can be indirectly determined by an elevated Kyn/Trp ratio (Schröcksnadel et al., 2006). Contrary to the effect of IDO induction, there have been cases with no increase in plasma Kyn under inflammatory circumstances, possibly explained through the upregulation of kynurenine 3- monooxygenase (KMO) (Badawy & Guillemin, 2019). Reduction of plasma tryptophan or increased Kyn - Trp ratio are described during viral, bacterial and parasitic intracellular infections (Boasso et al., 2007; Moreau et al., 2005; Murray, 2003; Silva et al., 2002) or experimentally induced inflammation (Melchior et al., 2004). Increased tryptophan utilization is also reported during other situations of long-lasting immune activation such as cancer (Denz et al., 1993; Liu et al., 2010), acquired immunodeficiency syndrome (AIDS) (Huengsberg et al., 1998), major trauma (Pellegrin et al.,

2005) and autoimmune, inflammatory diseases (e.g. MS) (Wolf et al., 2004). Metabolic disorders of the Kyn pathway are linked to the pathogenesis and progression of various chronic diseases. Dysregulations were discovered in diseases involving the central nervous system (CNS) (e. g. Multiple Sclerosis, Alzheimer's disease or Parkinson's disease (Campbell et al., 2014; Vécsei et al., 2013) and several internal pathologies (e.g. diabetes mellitus (Cervenka et al., 2017) and cancer (Platten et al., 2019).

Acute and chronic exercise have been shown to have strong influence on Trp metabolism (Metcalfe et al., 2018). Systematic physical activity and exercise interventions are assumed to have anti-inflammatory effects depending on the exercise modality, thus potentially reducing the risk and progress of several chronic diseases. Modifications in the kynurenine pathway may signify a link between inflammatory responses following acute exercise and chronic anti-inflammatory properties, such as increased levels of T_{regs} (Koliamitra et al., 2019). T_{regs} consist of a subpopulation of T cells that modulates the immune system, sustains tolerance to self-antigens, and prevents autoimmune diseases. The mechanisms under which this tolerance is mediated are not well understood, but there are findings that have connected tryptophan catabolism through the kynurenine metabolic pathway as one of many mechanisms involved. The enzymes that break down tryptophan through this pathway are found in numerous cell types, including cells of the immune system (Moffett & Namboodiri, 2003). Some of these enzymes are induced by immune activation like IDO. Various studies support the idea that, in addition to defense against pathogens, IDO participates in the regulation of T cell responses (Munn et al., 1999). It has been speculated that expression of IDO in antigen presenting cells of the immune system may control T_{regs} (Terness et al., 2002). T_{regs} are immunosuppressive and generally suppress or downregulate induction and proliferation of effector T cells (Bettelli et al., 2006). Adding all these facts it is clearly implied that tryptophan breakdown is essential for sustaining aspects of immune tolerance.

In this content, two original investigations were conducted on the effect of chronic and acute exercise on Kyn Pathway in persons with MS and healthy women. Supplementary aim of this thesis was to investigate any possible connection with regulatory T cells and the Kyn pathway influence. Additionally, a review screening the scientific background on exercise effects on Kyn Pathway on human subjects was conducted.

2. Scientific Background

2.1. Tryptophan metabolism

Tryptophan is mainly obtained through nutrition; chicken, eggs, cheese, fish and peanuts are examples of nutrients known to be high in this amino acid. Much of Trp stays in the guts whereas the remaining is transferred to the brain, heart and skeletal muscle (Agudelo et al., 2014; Cervenka et al., 2017). Moreover, only free plasma Trp can run through the blood brain barrier via large amino acid transporters (LAT) and then be metabolized into neuro-active substances (Agudelo et al., 2014). The level of free plasma Trp is, however, dependent on the albumin binding rate. This binding, useful for Trp body transport, can be altered with albumin levels and with the binding sites availability (Curzon et al., 1973). Definitely, free fatty acids can also be bound to albumin, subsequently lowering the Trp binding rate and increasing free plasma Trp levels (Curzon et al., 1973).

The TDO and its isoenzymes indoleamine 2, 3 dioxygenase 1 and 2 (IDO 1 + IDO 2) catalyze the degeneration of Trp to Kyn, thus demonstrating the very first step of the Kyn pathway. In contrast to TDO, which is mainly expressed in hepatic tissue and primarily stimulated by Trp itself or glucocorticoids (Badawy, 2017), IDO can be expressed in almost all types of human cells (Larkin et al., 2016). IDO1 and IDO2 have been reported to differ in respect to the level of expression, the effects on peripheral blood metabolites and the enzyme activity in response to specific physiological conditions. However, knowledge about the more recently discovered IDO2 is still weak and the differential roles of both enzymes are not fully comprehended yet (Munn & Mellor, 2013; Prendergast et al., 2018).

Activation of TDO demands high levels of cortisol (Viru et al., 2005). Otherwise, IDO whose activity is negligible under basal conditions, dramatically increases under the presence of inflammatory signals (Fuchs et al., 1990). It remains certain that IDO is enhanced by pro inflammatory cytokines such as IFN-y (Fuchs et al., 1990) but also TNF-α, IL-1β, IL-6 and IL-8 (Peake et al., 2015). The enzymes' (IDO and TDO) activity is up-regulated when inflammatory and stress markers are produced and the Kyn pathway is often over-activated in various pathologies (Chen & Guillemin, 2009). Since the last 20 years, the pathogenesis and

progression of various chronic diseases have been linked to metabolic disturbances of the Kyn pathway. Dysregulations were discovered in diseases involving the CNS (e. g. Multiple Sclerosis, Alzheimer's disease or Parkinson's disease (Campbell et al., 2014; Vécsei et al., 2013) and several internal pathologies (e.g. diabetes mellitus (Cervenka et al., 2017) and cancer (Platten et al., 2019).

The Kyn pathway metabolites also affect CNS homeostasis. Kyn itself can either be converted to the neuroprotective kynurenic acid (KYNA) or (over several intermediate steps) to quinolinic acid (QA), which is closely linked to neuronal excitotoxicity. QA is an endogenous neurotoxin of the glutamate N-methyl- D aspartate (NMDA) receptor shown to be neurotoxic, excitotoxic, cytotoxic and to have oxidative action (Meier et al., 2016; Tavares et al., 2002). On the other hand, KYNA is synthesized from Kyn and is assumed to have neuro-protective and anticonvulsant effect (Birch et al., 1988). Within the CNS, KYNA and QA act as antagonist and agonist of NMDA receptor activation respectively, thereby mediating either neuronal protection or excitotoxicity. In detail, KYNA has been described to act as NMDA receptor inhibitor, either at the strychnine-insensitive glycine-binding site (at lower concentrations) or at the glutamate site (at higher concentrations) (Chen & Guillemin, 2009). Additionally, KYNA is also able to inhibit α7 nicotinic acetylcholine receptors (nAChRs) that are expressed by glutamatergic axon terminals and involved in high glutamate release (Vécsei et al., 2013). Since NMDA receptor mediated excitotoxicity characterizes a common pathological mechanism in different neurodegenerative diseases (Vécsei et al., 2013), the antiglutamatergic properties of KYNA may have beneficial effects on neurodegenerative procedures. In regards to QA, the predominant and most-investigated neurotoxic effect is undoubtedly its activation of the NMDA receptor, leading to excitotoxicity. Nevertheless, several other neurotoxic aspects of QA have been labelled. These characteristics include the inhibition of glutamate uptake by astrocytes, the generation of reactive oxygen intermediates or the suppression of astroglial glutamine synthetase (Guillemin, 2012; Vécsei et al., 2013), emphasizing its strong association with CNS damage. It is interesting that neurons in certain brain regions, specifically the hippocampus, striatum and neocortex, seem to be more susceptible to QA than others (Guillemin, 2012).

The homeostasis of this neuroactive branch largely depends on the activity of the enzymes kynurenine aminotransferases (KATs) and KMO, which catalyze the degradation of Kyn to

KYNA or QA, respectively. More precisely, the conversion to KA can be mediated by four different isoforms of KAT (KAT 1-4), whereas the conversion to QA proceeds through two metabolic intermediate products, namely the 3-hydroxykynurenine (3-HK) and 3-hydroxy-anthranilic acid (3-HAA). Under non-pathological conditions, the vast majority of Kyn is metabolized to QA, yielding the preferred end product nicotinamide adenine dinucleotide (NAD^+), which is highly relevant for oxidative energy production. Based on specific environmental conditions or if higher levels of Trp or Kyn are present, the metabolic flux towards KYNA can increase (Cervenka et al., 2017). Regarding the neurotoxic potential along the Kyn pathway, it is not unexpected that central and/or peripheral dysregulations in metabolites and enzyme activities are associated with various neurodegenerative disorders, such as Alzheimer's disease, Huntington's disease or MS (Vécsei et al., 2013). Taking into consideration all these neurological diseases, an over-activated Kyn pathway leading to an accumulation of QA in the CNS as well as a decrease in the neuroprotective KYNA has been universally linked to pathogenesis or disease progression (Tan et al., 2012; Vécsei et al., 2013). Pathological QA accumulation encourages neurodegenerative processes involving neuro-inflammatory environment, a fact that assures CNS damage. As a consequence, KMO-Inhibitors signify a common therapeutic drug target in several neurological disorders (Vécsei et al., 2013).

Regarding the general physiology of the Kyn Pathway in Tryptophan metabolism and its end products, Trp is metabolized through three distinct biochemical pathways qualifying for protein and serotonin synthesis. The Kyn pathway starts with the enzymes IDO1, IDO2 or TDO catalyzing L-Trp into N-formylkynurenine. Following, kynurenine formamidase turns N-formylkynurenine into L-Kyn and formic acid. Kyn is then either transformed into anthranilic acid by kynureninase or altered into L-hydroxykynurenine by kynurenine hydroxylase. Anthranilic acid hydroxylates resulting in the production of L-hydroxykynurenine. L-hydroxykynurenine is accordingly transformed to 3-hydroxyanthranilic acid by Kynureninase and then into aminocarboxymuconic semialdehyde by hydroxyanthranilate dioxygenase. The semialdehyde produces quinolinic acid and aminomuconic semialdehyde. The last is followingly transformed into picolinic acid or glutaryl-coenzyme A (CoA) that is metabolized in the tricarbonic acid cycle and terminal oxidation (Acovic et al., 2018). (Figure 1)

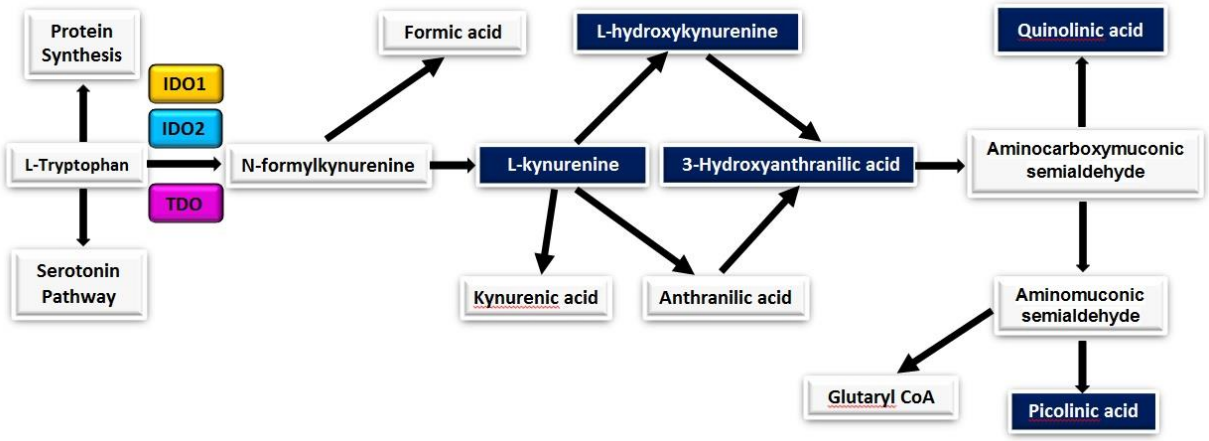

Figure 1. The Kyn Pathway of TRP Metabolism and its end products.

2.1.1. Kyn Pathway, Immune System Interactions and Regulatory T cells

Peripheral mononuclear blood cells (PBMCs) have been reported to be possible producers of IDO (Jones et al., 2015). Based on immune homeostasis, IDO activity is suspected to be constant and the conversion of Trp to Kyn is almost entirely mediated by TDO under basal conditions. In contrast, local and systemic inflammatory stimuli provoke a dramatic increase in IDO1 activity (Platten et al., 2019). In specific, elevated levels of Interferon-gamma (IFN-y) (Fuchs et al., 1990), but also high levels of Interleukin (IL)-6 and other pro-inflammatory cytokines (e.g. TNF-α, IL-1β, IL-8) seem to be significant stimuli for enhanced IDO activity (Peake et al., 2015), resulting in an increased Trp degradation. Specifically, IL- 6 is highly connected with cortisol levels which appear to regulate innate immune cell function (Gleeson, 2007). Kyn itself possesses immunomodulatory effects comprising the suppression of cytotoxic T-cell and natural killer (NK) -cell activity (Mándi & Vécsei, 2012; Munn et al., 1999; Munn & Mellor, 2013) and mediating the differentiation of T_{regs} (Chung et al., 2009). Evidence suggests that the IDO1-mediated adaptation of Kyn activates the aryl hydrocarbon receptor (AhR), which plays a key role in T-cell differentiation (Mezrich et al., 2010).

KYNA has been able to weaken inflammation caused by different stimuli (e.g. lipopolysaccharide stimulus- LPS) after investigation on various leukocyte cell types. In that case, KYNA diminishes tumor necrosis factor (TNF) expression (Steiner et al., 2014; Tiszlavicz et al., 2011; J. Wang et al., 2006; Wirthgen et al., 2014) and interleukin 4 release in T-cell receptor stimulated invariant natural killer-like T cells (iNKT) (Fallarini et al., 2010).

KYNA is a ligand of the G-protein coupled receptor 35 (GPR35) (J. Wang et al., 2006) and the AhR receptors (DiNatale et al., 2010; Platten et al., 2015). High levels of KYNA are formed under various inflammatory and tumor diseases, a fact showing that under these circumstances KYNA levels are plenty in order to activate these receptors (Platten et al., 2015). Except for its role in GRP35- and AhR-mediated signals, KYNA plays a significant role also as an antioxidant and reactive oxygen species (ROS) scavenger (Lugo-Huitrón et al., 2011; Pérez-González et al., 2015). This fact proves that KYNA acts preventing tissue damage generated by exceeding inflammation. Additionally, generation of the KYNA synthesis part of Trp metabolism may also be related to the synthesis of other Trp metabolites, such as serotonin or melatonin. Serotonin and melatonin act like immune regulators possibly able to

influence immune response (Reiter et al., 2006; Shajib & Khan, 2015). A number of immunoregulatory functions have been credited to serotonin, for example serotonin stimulates monocytes and lymphocytes and hence influences the secretion of cytokines. T-cell activation can also be mediated by serotonin (Arreola et al., 2015; Herr et al., 2017). Melatonin enhances both innate and cellular immunity. It stimulates the production of NK cells and cluster of differentiation 4 (CD4+) cells and inhibits CD8+ cells (Srinivasan et al., 2005). Their sufficiency can be challenged by KYNA production through the reduction of the necessary substrate Trp, or by direct inhibition of their synthesis, or induction of their degradation (Wirthgen et al., 2018).

2.1.2. Kyn Pathway and MS

MS is a demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are injured. This damage disrupts the ability of parts of the nervous system to transmit signals, following a range of signs and symptoms, including physical, mental, and sometimes psychiatric problems (Compston & Coles, 2008). MS takes several forms, with new symptoms either occurring in isolated attacks (relapsing forms) or building up over time (progressive forms)(Lublin & Reingold, 1996). Between attacks, symptoms may totally disappear; however, permanent neurological problems often remain, especially with the advancement of the disease (Lublin & Reingold, 1996). RRMS is characterized by unpredictable relapses followed by periods of months to years of relative quiet (remission) with no new signs of disease activity. Deficits that occur during attacks may either resolve or leave remaining problems, the latter in about 40% of attacks and being more common the longer a person has had the disease (Compston & Coles, 2008; Tsang & Macdonell, 2011). This describes the initial course of 80% of individuals with MS (Compston & Coles, 2008). SPMS occurs in around 65% of those with initial relapsing-remitting MS, who eventually have progressive neurologic decline between acute attacks without any definite periods of remission (Compston & Coles, 2008; Lublin & Reingold, 1996).

Chronically elevated Kyn/Trp ratios have been reported in persons with MS (Lim et al., 2017), although alterations in Trp metabolism seem to be dependent on the subtype of disease (Aeinehband et al., 2016). High L-kynurenine and N-formyl-kynurenine levels measured in the serum of persons with MS seem to reflect IDO activation (Sadowska-Bartosz et al., 2014). Additionally, increased L-kynurenine/Trp ratio was detected after handling with INF-_, involving IDO activation as a potential method of action of INF-_ products which are widely used for MS treatment (Amirkhani et al., 2005). Before treatment, IDO expression in PBMCs was analogous in acute MS relapse to that shown in healthy controls. During relapse and remission there has been increased and decreased global AhR activity reported in the serum, indicating the role of the endogenous AhR agonist L-kynurenine (Rothhammer et al., 2017). During relapses in RRMS patients, KYNA levels and KAT activity in the red blood cells (RBC) seem to increase (Hartai et al., 2005). All these facts constitute high IDO activation and downstream kynurenine metabolism during acute inflammatory aggravations in MS.

2.2. The Kyn pathway and exercise

This chapter is replaced by the first publication, which is summarized below. The original article is found in the appendix.

Publication 1:

Physiology and Behavior: 2018 194:583-587
Acute and chronic effects of exercise on the Kynurenine Pathway in humans – A brief review and future perspectives

Alan J Metcalfe#, Christina Koliamitra#, Wilhelm Bloch and Philipp Zimmer

#Author's shared first authorship

This brief review investigated the effect of acute and chronic exercise interventions on Trp and Kyn metabolism in healthy and diseased populations. It revealed that acute physical exercise appears to decrease levels of Trp and increase levels of Kyn. This effect appears to be consistent across both healthy and diseased populations. In contrast, according to studies that have examined the chronic effect of exercise and the relationship between different modes of exercise on levels of Trp and Kyn, results seem to be contradictory (Table 1).

Table 1: Effect of acute and chronic exercise intervention on markers of Trp metabolism and the Kyn pathway in healthy and diseased human exercise performance studies.

Author/s	Participants	Exercise Intervention	Outcome measurements
		Acute Exercise	
(Strasser et al., 2016)	33 trained athletes (17 women/16 men)	20 min maximal cycling time-trial	↑ KYN ↓ TRP

Author/s	Participants	Exercise Intervention	Outcome measurements
(Schlittler et al., 2016)	18 active males n = 9 active males (non-endurance) n = 9 active males (endurance)	1. 150-km road cycling time trial 2. Half marathon run 3. 100 drop jumps	↑ KAT ↑ KYNA ↔ KAYA
(Lewis et al., 2010)	25 healthy participants	Marathon run	↑ KYNA
(Bansi et al., 2018)	57 Multiple sclerosis *n* = 33 RRMS *n* = 24 SPMS	1. HIT 2. Continuous training	↓ TRP (RMMS only) ↔ KYN
(Mudry et al., 2016)	n = 12 Normal glucose tolerance *n* = 12 Type 2 diabetes	Cycling 30 min at 85% heart rate max	↓ TRP
	Chronic Exercise		
(Millischer et al., 2017)	117 mild-to-moderate depression	12-weeks, 3 groups (3x60 min/week): 1. Light (yoga) 2. Moderate (aerobics) Intensity (aerobics + strength)	↔ KYN ↔KYNA
(Hennings et al., 2013)	38 major depression n = 27 somatisation syndrome n = 48 healthy controls	7-days activity (30min/day low -intensity physical activity)	↔ KYN ↓TRP
(Küster et al., 2017)	17 risk of dementia	10-weeks physical training	↔ KYN ↔KYNA
(Bansi et al., 2018)	57 Multiple sclerosis *n* = 33 RRMS *n* = 24 SPMS	3-weeks, 1. HIT 2. Continuous Training	↔ KYN ↓TRP (RMMS only)
(Strasser et al., 2016)	29 trained athletes n = 14 probiotic group n = 15 placebo group	12 weeks, endurance training	↓TRP ↑ KYN
(Melancon et al., 2014)	16 healthy senior men	16-weeks aerobic training (45 min treadmill 3x/wk, moderate intensity)	↑ TRP availability

3. Questions

1. Does acute exercise exposure activate the Kyn Pathway in middle aged healthy female subjects? Is there a connection between Kyn pathway activation and regulatory T cells?

2. Does acute and chronic exercise exposure affect Kynurenine Pathway in patients with secondary progressive and relapsing remitting multiple sclerosis?

4. Methods and Results

The methods and results section is replaced by a summary of the publications underlying this thesis. The original publications are included in the appendix.

Publication 2:

Journal of Sports Science and Medicine 2019 18: 669-673

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

Christina Koliamitra, Florian Javelle, Niklas Joisten, Alexander Shimabukuro-Vornhagen, Wilhelm Bloch, Alexander Schenk and Philipp Zimmer

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 in-creased levels of T_{regs}. Here, we hypothesized 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 immuno-sorbent 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 re-search is needed to clarify to what extend acute exercise-induced activations of the kynurenine pathway contribute to T_{reg} differentiation.

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Persons with secondary progressive and relapsing remitting multiple sclerosis reveal different responses of tryptophan metabolism to acute endurance exercise and training

Jens Bansi#, Christina Koliamitra#, Wilhelm Bloch, Niklas Joisten, Alexander Schenk, Matthew Watson, Jan Kool, Dawn Langdon, Ulrik Dalgas, Jürg Kesselring, Philipp Zimmer

#Authors share first authorship

Disturbances in Tryptophan metabolism play a crucial role in MS. Exercise is suspected to counteract the progress of MS and its side effects. Current research suggests alterations of Tryptophan metabolism in healthy individuals in response to exercise. We investigated the influence of acute aerobic exercise and training on Tryptophan metabolism in 57 in persons with RRMS (n=33) and SPMS (n=24) MS. Serotonin increased after training, whereas the kynurenine pathway was only activated in persons with RRMS. Further research is warranted to investigate whether these changes are associated with clinical measures (e.g. depressions and immune function).

5. Discussion

In this part, the questions raised above, are discussed with regards to the results presented in the studies as well as in the context of the current research literature.

1. Does acute exercise exposure activate the Kyn Pathway in middle aged healthy female subjects? Is there a connection between regulatory T cells?

This publication (Appendix, p.40) consists the first time that acute endurance exercise has been shown to considerably activate the Kyn pathway in middle aged healthy women. Endurance capacity –as an indirect marker of fitness level- has been reported to be positively correlated with T_{reg} levels in this age group.

In the first publication of this thesis mentioned in the introduction (p.9), it has been reported that a single session of exhaustive endurance exercise is capable of activating the kynurenine pathway (Metcalfe et al., 2018), a fact that is justified here as well by the increased kynurenine and Kyn/Trp ratio of the sample examined. Possible reasons for the increased ratio could be a short-term inflammatory signal (Wang et al., 2015) - in this case triggered by acute exercise- initiating the Kyn pathway. Particularly, serum levels of pro-inflammatory cytokines, such as Interleukin-1 and Interleukin-6 increase in response to acute exercise (Fischer, 2006; Pedersen & Saltin, 2015; Walsh et al., 2011). Additionally, acute exercise is assumed to modify blood cortisol and free tryptophan concentrations (O'connor & Corrigan, 1987; Strasser, Geiger, Schauer, Gatterer, et al., 2016). Both aspects have previously been shown to be activators of TDO, a consecutive expressed liver-specific isoenzyme of IDO (Badawy, 2017). However, we did not observe any significant change in Trp levels in opposition to Strasser et al. who have shown a significant decrease of Trp after exercise. Although, it is worth to underline that both studies are hardly considered equivalent since the participants (older women vs. young athletes) and exercise protocols/duration (CPET: 15.8 minutes vs. CPET + 20 minutes maximum time trial >30 minutes over-all) strongly differ. Regarding cortisol, studies uncover inconsistent results on acute exercise-induced effects (Hayes et al., 2015). Consequently, it cannot be dismissed that the observed increase in kynurenine and Kyn/Trp ratio is also driven by an induction of TDO.

Opposing to the elevated Kyn/Trp ratio, results of this publication reveal no changes in the Trp/Serotonin (SER) ratio. This fact shows that the Kyn pathway is activated by acute exercise but there is no equivalent influence on the peripheral serotonin metabolism.

Previously, enhanced endurance capacity has been discovered to correlate with increased T_{reg} numbers and proportions in athletes (Weinhold et al., 2016). Corresponding to these results, this publication revealed an equivalent association. Therefore, the findings of this publication support that repeated short term increases of kynurenine levels, as it occurs after every session of acute exercise, may result in a long term increase in T_{regs}. However, it should be taken into consideration that the discovered connection between acute increases in biomarkers - in particular kynurenine - and long-term adaptions – in particular induction of T_{regs} - seems mainly difficult to justify in longitudinal in vivo studies. The products of tryptophan metabolism, such as indoleamine 2, 3-dioxygenase, kynurenine, quinolinic acid, and melatonin, may improve immunity in an organism and induce anti-inflammatory responses. The immune tolerance processes mediated by tryptophan metabolites are not well understood. Recent studies have reported that the enzymes that break down tryptophan through the kynurenine metabolic pathway are found in numerous cell types, including immunocytes. Moreover, some tryptophan metabolites have been shown to play a role in the inhibition of T lymphocyte proliferation, elevation of immunoglobulin levels in the blood, and promotion of antigen-presenting organization in tissues (Bai et al., 2017).

To conclude, answering the first question of this thesis, acute exercise exposure is able to activate the Kyn Pathway in middle aged healthy women. This group consists of a population at risk for several diseases (Tice et al., 2006), although it is a rather small sample size that was not compared to a control group. Kyn pathway parameters in this study were measured with enzyme-linked immunosorbent assay (ELISA) although the gold standard method is ultra-high-performance liquid chromatography (HPLC) coupled to electrospray ionization triple–quadrupole mass spectrometry and should be prioritized by future studies when possible. Furthermore, certainly more research is necessary to shed light on whether this activation is a consequence of changes in IDO/TDO initiation and activity. Further research should be performed regarding gene expression, protein analysis and an extensive variety of markers such as KYNA or QA to cover the neuroregulatory bench of the Kyn pathway, perhaps also in different clinical population groups. It is remarkable to mention that this age

group is related with low-grade systemic inflammation, a state in which tissues express high levels of inflammatory factors and immune cell infiltration (León-Pedroza et al., 2015). Inflammation aims to restore tissue homeostasis after injury or infection. Activation of Trp metabolism along the Kyn pathway prevents hyperinflammation and induces long-term immune tolerance (Sorgdrager et al., 2019). Systemic Trp and Kyn levels change upon aging (Sorgdrager et al., 2019), a fact that should be further investigated in similar middle-aged groups. Additionally, there is connection - previously described also in athletes - between exercise and T_{reg} quantities (as a marker of anti-inflammatory potential) in older women. Further investigations are considered essential to strongly improve the statement of this publication that an increase in T_{regs} is encouraged by repetitive exercise induced increases in kynurenine levels and also investigate it on a mechanistic level. It should be taken into consideration that this connection between increase in biomarkers like Kyn and long-term adaptations like the induction of T_{regs} appears hard to prove in longitudinal in vivo studies. There are two theories that have been proposed to explain how tryptophan catabolism facilitates immune tolerance. One theory suggests that tryptophan breakdown suppresses T cell proliferation by dramatically reducing the supply of this critical amino acid. The other theory proposes that the downstream metabolites of tryptophan catabolism act to suppress certain immune cells, probably by pro-apoptotic mechanisms. Merging these disparate views is crucial to comprehend immune-related tryptophan catabolism and the roles it plays in immune tolerance (Moffett & Namboodiri, 2003). High T_{reg} levels offer a strong anti-inflammatory potential as defence against undesired immune reactions (Lei et al., 2015), cardiovascular diseases and neurological diseases, a fact that can prove essential for any risks related to this age population.

2. How does acute and chronic exercise exposure affect Kynurenine Pathway in patients with secondary progressive and relapsing remitting multiple sclerosis?

Patients with RRMS are patients who face relapses of MS and periods of stability in between relapses. Patients with SPMS go through phases of relapse followed by remission at first, but this later gives way to progressive disease, worsening symptoms with few or absence of remission. This MS subgroup analysis was conducted since Trp metabolism alterations seem to be dependent on the subtype of disease (Aeinehband et al., 2016). Lower Kyn levels in both SPMS and RRMS patients were connected to an increased cardiorespiratory fitness level. Since IDO activity is known to be up-regulated by inflammatory stimuli, these findings support the evidence that frequent physical exercise has anti-inflammatory properties (Hojman, 2017; Weinhold et al., 2016). Additionally, regular exercise seems to increase KAT expression in skeletal muscle leading to an increased Kyn breakdown in individuals with a higher cardiopulmonary fitness (Agudelo et al., 2014). Nevertheless, in this publication elevated resting Kyn/Trp ratio and decreased Trp levels after high intensity training (HIT) and control training (CT) in RRMS patients is also detected. Kyn/Trp ratio constitutes a marker for disease activity. Elevated ratio during or after inpatient rehabilitation may mean higher demand of amino acids such as Trp since a Trp increase results in an elevated Kyn/Trp ratio. Another scenario is that Trp actually decreased due to a transmission to muscle tissue to cover the elevated need of amino acids for protein synthesis after training. In contrast to RRMS patients, the training interventions did not change Trp and Kyn/Trp ratio in patients with SPMS. These findings are in accordance with those of Millischer et al. who did not find any chronic alterations of Trp metabolism in persons with major depression (Millischer et al., 2017). Different responses of Trp metabolism could be argued by the already strongly decreased Trp levels in patients with SPMS or by their almost significantly increased cardio-pulmonary fitness.

As previously shown in healthy subjects, acute exhaustive exercise activates the Kyn pathway in RRMS patients (Strasser et al., 2016). According to evidence, this activation is mainly driven by strongly elevated levels of inflammatory mediators such as IL-6 and IFN-gamma during and directly after acute exercise, thereby activating IDO (Schröcksnadel et al., 2006; Walsh et al., 2011). Once more, persons with SPMS did not reveal the expected response of Trp metabolism. Further research has to reveal if this lack of response is due to

the decreased Trp baseline levels or if this may be influenced by their increased physical fitness at baseline.

Regarding 5-hydroxytrypramine receptors (5HT), patients with RRMS and SPMS showed similar responses to acute exercise and training. Contrary to results suggesting an observed increase in 5HT after acute exercise, there was no statistical significance found. However, significantly elevated 5HT levels were found in both patient groups after the three-week interventions. In healthy persons, acute exercise resulted in increased serum 5HT levels (Zimmer et al., 2016), a fact that is probably owed to a shear stress-induced release of 5HT of platelets (Lu et al., 2013).

Although peripheral 5HT is not capable of crossing the blood brain barrier, it has been proven that peripheral and central 5HT levels are closely connected (Audhya et al., 2012). There is need of further research to examine the exercise-induced increases in 5HT. Since data in RRMS patients reveal a negative correlation between 5HT levels and Kyn, it seems reasonable that more Trp is metabolized to 5HT than is degraded within the Kyn pathway.

To sum these findings up, their clinical relevance regarding persons with MS requires to get clarified under further investigation. To this context, animal models could provide new information on direct effects of exercise-induced alterations in Trp metabolism on the central nervous system. On one hand this may suggest that the reduction of potential neurotoxic agents (QA and KYNA) as a result of systemic and central decrease in Kyn and its metabolites could prove advantageous. Kyn pathway parameters have a strong association with MS subtype, connecting with disease severity forms since KYNA can act as neuroprotective and QA as a neurotoxic marker (Lim et al., 2017). There is a possibility that evaluating the Kyn pathway metabolites into detail may explain the transition from RRMS subgroup to a SPMS disease type and may provide potential therapeutic insights in decelerating neurodegeneration in MS. On the other hand, exercise-caused higher Kyn levels could result in immune system adaptations (for example T_{regs} increase) which are closely associated to respond to chronic inflammation and an immune over-activation (as they act in MS). The analysis and investigation on Kyn pathway metabolomics and especially KYNA and QA which was here missing, seems essential to drive useful conclusions on the metabolic flux and further comprehend any neurotoxicity dimensions. Additional investigation in this research area should also take into consideration other MS subtypes, more promising

exercise models (for example resistance exercise) and a wider variety of Trp metabolites and enzymes such as KYNA, QA and KAT not only as blood based biomarkers but also compared to those in the CNS. The range of metabolites of tryptophan in the CNS, including serotonin, KYNA, QA and melatonin points towards a need for integrative approaches to understand the functional consequences of tryptophan metabolism in the CNS (Ruddick et al., 2006). Kynurenines play an anti-inflammatory role also in CNS disorders such as Huntington's disease, Alzheimer's disease and MS, in which signs of inflammation and neurodegeneration are involved (Huang et al., 2020).

To conclude, the question stated is confirmed demonstrating the different responses of chronic and acute exercise on Tryptophan metabolism in SPMS and RRMS. This is the first study to validate the different responses of Trp metabolism to acute and chronic exercise in these groups, shaping new questions in this area. It is suggested for future studies to estimate Trp metabolism through the gold standard methods of HPLC coupled with MS. Moreover, there is demand for additional research on whether the demonstrated modifications have impact on targets of Trp metabolites, such as immune function, depression and cognitive performance in persons with MS.

6. Conclusion and Outlook

The first study of this thesis is a systematic review collecting any existing knowledge and scientific background regarding acute and chronic effects of exercise on Tryptophan metabolism. Going through all the populations examined in this topic, it seemed obvious that there are age populations as well as neurological patients missing, a fact that should be considered in subsequent publications aiming to shed light on the responses of middle aged women and persons with MS. The studies presented are the first to investigate the effects of endurance exercise on Trp metabolism in middle aged women and persons with MS. They showed that acute exercise exposure is able to activate the Kyn Pathway in middle aged healthy women, whereas patients from different MS subgroups show different responses when exposed to chronic and acute exercise as well. Both studies reveal several hints that future research should focus on, regarding T_{regs} and their anti-inflammatory potential against health challenges related to this age group and how different metabolism responses in MS subgroups can constitute a key to reveal new prevention or therapeutic insights for different subforms of neurological diseases. Acute activation resulting in higher Kyn/Trp ratio and increased T_{regs} proportions can potentially provide significant immune system response through neuroprotection or reasonable immunomodulatory effects. In the future, different exercise modalities in terms of intensity and duration, but also endurance and strength training should be considered in order to further investigate different Trp metabolism responses. Further studies should examine the Kyn pathway responses in terms of chronic effects of exercise since there is poor scientific input on this matter. There should be an understanding on how exercise can target increase in T_{reg} levels and how middle aged groups can profit from their anti-inflammatory immune responses on cardiovascular or neurological diseases. Additional insights should be provided in the future regarding any neuroprotective properties of KYNA in metabolic flux and T_{regs} proportions induced by regulating AhR ligands. Further studies need to follow, which should shed light on how Kyn pathway metabolomics respond both in blood and central nervous system in different types of neurological diseases. Combinations of chronic and acute training according to different patient characteristics could reveal valuable information of mechanisms that remain unclear so far.

7. References

Acovic, A., Gazdic, M., Jovicic, N., Harrell, C. R., Fellabaum, C., Arsenijevic, N., & Volarevic, V. (2018). Role of indoleamine 2,3-dioxygenase in pathology of the gastrointestinal tract. In *Therapeutic Advances in Gastroenterology* (Vol. 11, p. 1756284818815334). SAGE Publications Ltd. https://doi.org/10.1177/1756284818815334

Aeinehband, S., Brenner, P., Ståhl, S., Bhat, M., Fidock, M. D., Khademi, M., Olsson, T., Engberg, G., Jokinen, J., Erhardt, S., & Piehl, F. (2016). Cerebrospinal fluid kynurenines in multiple sclerosis; relation to disease course and neurocognitive symptoms. *Brain, Behavior, and Immunity*, *51*, 47–55. https://doi.org/10.1016/j.bbi.2015.07.016

Agudelo, L. Z., Femenía, T., Orhan, F., Porsmyr-Palmertz, M., Goiny, M., Martinez-Redondo, V., Correia, J. C., Izadi, M., Bhat, M., Schuppe-Koistinen, I., Pettersson, A. T., Ferreira, D. M. S., Krook, A., Barres, R., Zierath, J. R., Erhardt, S., Lindskog, M., & Ruas, J. L. (2014). Skeletal Muscle PGC-1a1 Modulates Kynurenine Metabolism and Mediates Resilience to Stress-Induced Depression. *Cell*, *159*(1), 33–45. https://doi.org/10.1016/j.cell.2014.07.051

Amirkhani, A., Rajda, C., Arvidsson, B., Bencsik, K., Boda, K., Seres, E., Markides, K. E., Vécsei, L., & Bergquist, J. (2005). Interferon-β affects the tryptophan metabolism in multiple sclerosis patients. *European Journal of Neurology*, *12*(8), 625–631. https://doi.org/10.1111/j.1468-1331.2005.01041.x

Arreola, R., Becerril-Villanueva, E., Cruz-Fuentes, C., Velasco-Velázquez, M. A., Garces-Alvarez, M. E., Hurtado-Alvarado, G., Quintero-Fabian, S., & Pavon, L. (2015). Immunomodulatory effects mediated by serotonin. In *Journal of Immunology Research* (Vol. 2015). Hindawi Publishing Corporation. https://doi.org/10.1155/2015/354957

Audhya, T., Adams, J. B., & Johansen, L. (2012). Correlation of serotonin levels in CSF, platelets, plasma, and urine. *Biochimica et Biophysica Acta - General Subjects*, *1820*(10), 1496–1501. https://doi.org/10.1016/j.bbagen.2012.05.012

Badawy, A. A.-B. (2017a). Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. *International Journal of Tryptophan Research : IJTR*, *10*(1), 1178646917691938. https://doi.org/10.1177/1178646917691938

Badawy, A. A.-B. (2017b). Tryptophan availability for kynurenine pathway metabolism across the life span: Control mechanisms and focus on aging, exercise, diet and nutritional supplements. *Neuropharmacology*, *112*, 248–263. https://doi.org/10.1016/J.NEUROPHARM.2015.11.015

Badawy, A. A.-B., & Guillemin, G. (2019). The Plasma [Kynurenine]/[Tryptophan] Ratio and Indoleamine 2,3-Dioxygenase: Time for Appraisal. *International Journal of Tryptophan Research : IJTR*, *12*, 1178646919868978. https://doi.org/10.1177/1178646919868978

Bai, M., Liu, H., Xu, K., Oso, A. O., Wu, X., Liu, G., Tossou, M. C. B., Al-Dhabi, N. A., Duraipandiyan, V., Xi, Q., & Yin, Y. (2017). A review of the immunomodulatory role of dietary tryptophan in livestock and poultry. In *Amino Acids* (Vol. 49, Issue 1, pp. 67–74). Springer-Verlag Wien. https://doi.org/10.1007/s00726-016-2351-8

Bansi, J., Koliamitra, C., Bloch, W., Joisten, N., Schenk, A., Watson, M., Kool, J., Langdon, D., Dalgas, U., Kesselring, J., & Zimmer, P. (2018). Persons with secondary progressive and relapsing remitting multiple sclerosis reveal different responses of tryptophan metabolism to acute endurance exercise and training. *Journal of Neuroimmunology*, *314*, 101–105. https://doi.org/10.1016/j.jneuroim.2017.12.001

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Weiner, H. L., & Kuchroo, V. K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, *441*(7090), 235–238. https://doi.org/10.1038/nature04753

Birch, P. J., Grossman, C. J., & Hayes, A. G. (1988). Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor. *European Journal of Pharmacology*, *154*(1), 85–87. https://doi.org/10.1016/0014-2999(88)90367-6

Boasso, A., Vaccari, M., Hryniewicz, A., Fuchs, D., Nacsa, J., Cecchinato, V., Andersson, J., Franchini, G., Shearer, G. M., & Chougnet, C. (2007). Regulatory T-Cell Markers, Indoleamine 2,3-Dioxygenase, and Virus Levels in Spleen and Gut during Progressive Simian Immunodeficiency Virus Infection. *Journal of Virology*, *81*(21), 11593–11603. https://doi.org/10.1128/jvi.00760-07

Braidy, N., Guillemin, G. J., Mansour, H., Chan-Ling, T., & Grant, R. (2011). Changes in kynurenine pathway metabolism in the brain, liver and kidney of aged female Wistar rats. *The FEBS Journal*, *278*(22), 4425–4434. https://doi.org/10.1111/j.1742-4658.2011.08366.x

Campbell, B. M., Charych, E., Lee, A. W., & Möller, T. (2014). Kynurenines in CNS disease: Regulation by inflammatory cytokines. *Frontiers in Neuroscience*, *8*(8 FEB), 1–22. https://doi.org/10.3389/fnins.2014.00012

Cervenka, I., Agudelo, L. Z., & Ruas, J. L. (2017). Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. In *Science* (Vol. 357, Issue 6349). American Association for the Advancement of Science. https://doi.org/10.1126/science.aaf9794

Chen, Y., & Guillemin, G. J. (2009). Kynurenine pathway metabolites in humans: disease and healthy States. *International Journal of Tryptophan Research : IJTR*, *2*, 1–19.

Chung, D. J., Rossi, M., Romano, E., Ghith, J., Yuan, J., Munn, D. H., & Young, J. W. (2009). Indoleamine 2,3-dioxygenase-expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. *Blood*, *114*(3), 555–563. https://doi.org/10.1182/blood-2008-11-191197

Compston, A., & Coles, A. (2008). Multiple sclerosis. In *The Lancet* (Vol. 372, Issue 9648, pp. 1502–1517). https://doi.org/10.1016/S0140-6736(08)61620-7

Curzon, G., Friedel, J., & Knott, P. J. (1973). The effect of fatty acids on the binding of tryptophan to plasma protein. *Nature*, *242*(5394), 198–200. https://doi.org/10.1038/242198a0

Denz, H., Orth, B., Weiss, G., Herrmann, R., Huber, P., Wachter, H., & Fuchs, D. (1993). Weight loss in patients with hematological neoplasias is associated with immune system stimulation. *The Clinical Investigator*, *71*(1), 37–41. https://doi.org/10.1007/BF00210961

DiNatale, B. C., Murray, I. A., Schroeder, J. C., Flaveny, C. A., Lahoti, T. S., Laurenzana, E. M., Omiecinski, C. J., & Perdew, G. H. (2010). Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicological Sciences*, *115*(1), 89–97. https://doi.org/10.1093/toxsci/kfq024

Fallarini, S., Magliulo, L., Paoletti, T., de Lalla, C., & Lombardi, G. (2010). Expression of functional GPR35 in human iNKT cells. *Biochemical and Biophysical Research Communications*, *398*(3), 420–425. https://doi.org/10.1016/j.bbrc.2010.06.091

Fischer, C. P. (2006). Interleukin-6 in acute exercise and training: What is the biological relevance? In *Exercise Immunology Review* (Vol. 12, pp. 6–33).

Fuchs, D., Möller, A. A., Reibnegger, G., Stöckle, E., Werner, E. R., & Wachter, H. (1990). Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *Journal of Acquired Immune Deficiency Syndromes*, *3*(9), 873–876.

Gleeson, M. (2007). Immune function in sport and exercise. In *Journal of Applied Physiology* (Vol. 103, Issue 2, pp. 693–699). American Physiological Society. https://doi.org/10.1152/japplphysiol.00008.2007

Guillemin, G. J. (2012). Quinolinic acid, the inescapable neurotoxin. In *FEBS Journal* (Vol. 279, Issue 8, pp. 1356–1365). https://doi.org/10.1111/j.1742-4658.2012.08485.x

Hartai, Z., Klivenyi, P., Janaky, T., Penke, B., Dux, L., & Vecsei, L. (2005). Kynurenine metabolism in multiple sclerosis. *Acta Neurologica Scandinavica*, *112*(2), 93–96. https://doi.org/10.1111/j.1600-0404.2005.00442.x

Hayes, L. D., Grace, F. M., Baker, J. S., & Sculthorpe, N. (2015). Exercise-induced responses in salivary testosterone, cortisol, and their ratios in men: a meta-analysis. *Sports Medicine (Auckland, N.Z.)*, *45*(5), 713–726. https://doi.org/10.1007/s40279-015-0306-y

Hennings, A., Schwarz, M. J., Riemer, S., Stapf, T. M., Selberdinger, V. B., & Rief, W. (2013). Exercise affects symptom severity but not biological measures in depression and somatization - Results on IL-6, neopterin, tryptophan, kynurenine and 5-HIAA. *Psychiatry Research*, *210*(3), 925–933. https://doi.org/10.1016/j.psychres.2013.09.018

Herr, N., Bode, C., & Duerschmied, D. (2017). The Effects of Serotonin in Immune Cells. In *Frontiers in Cardiovascular Medicine* (Vol. 4, p. 48). Frontiers Media S.A. https://doi.org/10.3389/fcvm.2017.00048

Hojman, P. (2017). Exercise protects from cancer through regulation of immune function and inflammation. *Biochemical Society Transactions*, *45*(4), 905–911. https://doi.org/10.1042/BST20160466

Huang, Y. S., Ogbechi, J., Clanchy, F. I., Williams, R. O., & Stone, T. W. (2020). IDO and Kynurenine Metabolites in Peripheral and CNS Disorders. In *Frontiers in Immunology* (Vol. 11, p. 388). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2020.00388

Huengsberg, M., Winer, J. B., Compels, M., Round, R., Ross, J., & Shahmanesh, M. (1998). Serum kynurenine-to-tryptophan ratio increases with progressive disease in HIV-infected patients. *Clinical Chemistry*, *44*(4), 858–862. https://doi.org/10.1093/clinchem/44.4.858

Jones, S. P., Franco, N. F., Varney, B., Sundaram, G., Brown, D. A., De Bie, J., Lim, C. K., Guillemin, G. J., & Brew, B. J. (2015). Expression of the kynurenine pathway in human peripheral blood mononuclear cells: Implications for inflammatory and neurodegenerative disease. *PLoS ONE*, *10*(6), 1–18. https://doi.org/10.1371/journal.pone.0131389

Pellegrin, K., Neurauter, G., Wirleitner, B., Fleming, A. W., Peterson, V. M., & Fuchs, D. (2005). Enhanced enzymatic degradation of tryptophan by indoleamine 2,3-dioxygenase contributes to the tryptophan-deficient state seen after major trauma. *Shock*, *23*(3), 209–215. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L40293819

Koliamitra, C., Javelle, F., Joisten, N., Shimabukuro-Vornhagen, A., Bloch, W., Schenk, A., & Zimmer, P. (2019). Acute Exercise-Induced Activations of the Kynurenine Pathway Induce Reg-ulatory T-Cells on the Long-Term?-A Theoretical Frame Work Supported by Pilot Data. In *©Journal of Sports Science and Medicine* (Vol. 18). http://www.jssm.org

Küster, O. C., Laptinskaya, D., Fissler, P., Schnack, C., Zügel, M., Nold, V., Thurm, F., Pleiner, S., Karabatsiakis, A., Von Einem, B., Weydt, P., Liesener, A., Borta, A., Woll, A., Hengerer, B., Kolassa, I. T., & Von Arnim, C. A. F. (2017). Novel Blood-Based Biomarkers of Cognition, Stress, and Physical or Cognitive Training in Older Adults at Risk of Dementia: Preliminary Evidence for a Role of BDNF, Irisin, and the Kynurenine Pathway. *Journal of Alzheimer's Disease*, *59*(3), 1097–1111. https://doi.org/10.3233/JAD-170447

Larkin, P. B., Sathyasaikumar, K. V., Notarangelo, F. M., Funakoshi, H., Nakamura, T., Schwarcz, R., & Muchowski, P. J. (2016). Tryptophan 2,3-Dioxygenfase and Indoleamine 2,3-Dioxygenase 1 Make Separate, Tissue-Specific Contributions to Basal and Inflammation-Induced Kynurenine Pathway Metabolism in Mice. *Biochimica et Biophysica Acta*, *1860*(11), 2345–2354. https://doi.org/10.1016/j.trsl.2014.08.005.The

Lei, H., Schmidt-Bleek, K., Dienelt, A., Reinke, P., & Volk, H. D. (2015). Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners. *Frontiers in Pharmacology*, *6*(SEP), 2. https://doi.org/10.3389/fphar.2015.00184

Leklem, J. E. (1971). Quantitative aspects of tryptophan metabolism in humans and other species: a review. *The American Journal of Clinical Nutrition*, *24*(6), 659–672. https://doi.org/10.1093/ajcn/24.6.659

León-Pedroza, J. I., González-Tapia, L. A., Del Olmo-Gil, E., Castellanos-Rodríguez, D., Escobedo, G., & González-Chávez, A. (2015). Inflamación sistémica de grado bajo y su relación con el desarrollo de enfermedades metabólicas: de la evidencia molecular a la aplicación clínica. *Cirugia y Cirujanos*, *83*(6), 543–551. https://doi.org/10.1016/j.circir.2015.05.041

Lewis, G. D., Farrell, L., Wood, M. J., Martinovic, M., Arany, Z., Rowe, G. C., Souza, A., Cheng, S., Mccabe, E. L., Yang, E., Deo, R., Roth, F. P., Asnani, A., Rhee, E. P., Systrom, D. M., Semigran, M. J., Vasan, R. S., Carr, S. A., Wang, T. J., … Gerszten, R. E. (2010). Metabolic Signatures of Exercise in Human Plasma. *Science Translational Magazine*, *2*(33), 33ra37. https://doi.org/10.1126/scitranslmed.3001006

Lim, C. K., Bilgin, A., Lovejoy, D. B., Tan, V., Bustamante, S., Taylor, B. V, Bessede, A., Brew, B. J., & Guillemin, G. J. (2017). Kynurenine pathway metabolomics predicts and provides mechanistic insight into multiple sclerosis progression. *Scientific Reports*, *7*, 41473. https://doi.org/10.1038/srep41473

Liu, X., Shin, N., Koblish, H. K., Yang, G., Wang, Q., Wang, K., Leffet, L., Hansbury, M. J., Thomas, B., Rupar, M., Waeltz, P., Bowman, K. J., Polam, P., Sparks, R. B., Yue, E. W., Li, Y., Wynn, R., Fridman, J. S., Burn, T. C., … Scherle, P. A. (2010). Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. *Blood*, *115*(17), 3520–3530. https://doi.org/10.1182/blood-2009-09-246124

Lu, Q., Hofferbert, B. V, Koo, G., & Malinauskas, R. A. (2013). In vitro shear stress-induced platelet activation: sensitivity of human and bovine blood. *Artificial Organs*, *37*(10), 894–903. https://doi.org/10.1111/aor.12099

Lublin, F. D., & Reingold, S. C. (1996). Defining the clinical course of multiple sclerosis: Results of an international survey. In *Neurology* (Vol. 46, Issue 4, pp. 907–911). Lippincott Williams and Wilkins. https://doi.org/10.1212/WNL.46.4.907

Lugo-Huitrón, R., Blanco-Ayala, T., Ugalde-Muñiz, P., Carrillo-Mora, P., Pedraza-Chaverrí, J., Silva-Adaya, D., Maldonado, P. D., Torres, I., Pinzón, E., Ortiz-Islas, E., López, T., García, E., Pineda, B., Torres-Ramos, M., Santamaría, A., & La Cruz, V. P.-D. (2011). On the antioxidant properties of kynurenic acid: free radical scavenging activity and inhibition of oxidative stress.

Neurotoxicology and Teratology, *33*(5), 538–547. https://doi.org/10.1016/j.ntt.2011.07.002

Mándi, Y., & Vécsei, L. (2012). The kynurenine system and immunoregulation. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *119*(2), 197–209. https://doi.org/10.1007/s00702-011-0681-y

Meier, T. B., Drevets, W. C., Wurfel, B. E., Ford, B. N., Morris, H. M., Victor, T. A., Bodurka, J., Teague, T. K., Dantzer, R., & Savitz, J. (2016). Relationship between neurotoxic kynurenine metabolites and reductions in right medial prefrontal cortical thickness in major depressive disorder. *Brain, Behavior, and Immunity*, *53*, 39–48. https://doi.org/10.1016/j.bbi.2015.11.003

Melancon, M. O., Lorrain, D., & Dionne, I. J. (2014). Changes in markers of brain serotonin activity in response to chronic exercise in senior men. *Applied Physiology, Nutrition and Metabolism*, *39*(11), 1250–1256. https://doi.org/10.1139/apnm-2014-0092

Melchior, D., Sève, B., & Le Floc'h, N. (2004). Chronic lung inflammation affects plasma amino acid concentrations in pigs. *Journal of Animal Science*, *82*(4), 1091–1099. https://doi.org/10.2527/2004.8241091x

Metcalfe, A. J., Koliamitra, C., Javelle, F., Bloch, W., & Zimmer, P. (2018). Acute and chronic effects of exercise on the kynurenine pathway in humans – A brief review and future perspectives. *Physiology and Behavior*, *194*(July), 583–587. https://doi.org/10.1016/j.physbeh.2018.07.015

Mezrich, J. D., Fechner, J. H., Zhang, X., Johnson, B. P., Burlingham, W. J., & Bradfield, C. A. (2010). An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *Journal of Immunology (Baltimore, Md. : 1950)*, *185*(6), 3190–3198. https://doi.org/10.4049/jimmunol.0903670

Millischer, V., Erhardt, S., Ekblom, Ö., Forsell, Y., & Lavebratt, C. (2017). Twelve-week physical exercise does not have a long-lasting effect on kynurenines in plasma of depressed patients. *Neuropsychiatric Disease and Treatment*, *13*, 967–972. https://doi.org/10.2147/NDT.S131746

Moffett, J. R., & Namboodiri, M. A. (2003). Tryptophan and the immune response. In *Immunology and Cell Biology* (Vol. 81, Issue 4, pp. 247–265). John Wiley & Sons, Ltd. https://doi.org/10.1046/j.1440-1711.2003.t01-1-01177.x

Moreau, M., Lestage, J., Verrier, D., Mormede, C., Kelley, K. W., Dantzer, R., & Castanon, N. (2005). Bacille Calmette-Guérin inoculation induces chronic activation of peripheral and brain indoleamine 2,3-dioxygenase in mice. *The Journal of Infectious Diseases*, *192*(3), 537–544. https://doi.org/10.1086/431603

Mudry, J. M., Alm, P. S., Erhardt, S., Goiny, M., Fritz, T., Caidahl, K., Zierath, J. R., Krook, A., & Wallberg-Henriksson, H. (2016). Direct effects of exercise on kynurenine metabolism in people with normal glucose tolerance or type 2 diabetes. *Diabetes/Metabolism Research and Reviews*, *32*(30), 754–761. https://doi.org/10.1002/dmrr.2798

Munn, D. H., & Mellor, A. L. (2013). Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends in Immunology*, *34*(3), 137–143. https://doi.org/10.1016/j.it.2012.10.001

Munn, D. H., Shafizadeh, E., Attwood, J. T., Bondarev, I., Pashine, A., & Mellor, A. L. (1999). Inhibition of T Cell Proliferation by Macrophage Tryptophan Catabolism. *The Journal of Experimental Medicine*, *189*(9), 1363–1372. https://doi.org/10.1084/jem.189.9.1363

Murray, M. F. (2003). Tryptophan depletion and HIV infection: A metabolic link to pathogenesis. In *Lancet Infectious Diseases* (Vol. 3, Issue 10, pp. 644–652). Lancet Publishing Group. https://doi.org/10.1016/S1473-3099(03)00773-4

O'connor, P. J., & Corrigan, D. L. (1987). Influence of short-term cycling on salivary cortisol levels. *Medicine and Science in Sports and Exercise*, *19*(3), 224–228. https://doi.org/10.1249/00005768-198706000-00007

Peake, J. M., Della Gatta, P., Suzuki, K., & Nieman, D. C. (2015). Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. *Exercise Immunology Review*, *21*, 8–25.

Pedersen, B. K., & Saltin, B. (2015). Exercise as medicine - Evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scandinavian Journal of Medicine and Science in Sports*, *25*, 1–72. https://doi.org/10.1111/sms.12581

Pérez-González, A., Alvarez-Idaboy, J. R., & Galano, A. (2015). Free-radical scavenging by tryptophan and its metabolites through electron transfer based processes. *Journal of Molecular Modeling*, *21*(8), 213. https://doi.org/10.1007/s00894-015-2758-2

Platten, M., Nollen, E. A. A., Röhrig, U. F., Fallarino, F., & Opitz, C. A. (2019). Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. *Nature Reviews Drug Discovery*. https://doi.org/10.1038/s41573-019-0016-5

Platten, M., von Knebel Doeberitz, N., Oezen, I., Wick, W., & Ochs, K. (2015). Cancer immunotherapy by targeting IDO1/TDO and their downstream effectors. In *Frontiers in Immunology* (Vol. 6, Issue JAN, p. 673). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2014.00673

Prendergast, G. C., Malachowski, W. J., Mondal, A., Scherle, P., & Muller, A. J. (2018). Indoleamine 2,3-Dioxygenase and Its Therapeutic Inhibition in Cancer. *International Review of Cell and Molecular Biology*, *336*, 175–203. https://doi.org/10.1016/bs.ircmb.2017.07.004

Reiter, R. J., Calvo, J. R., Karbownik, M., Qi, W., & Tan, D. X. (2006). Melatonin and Its Relation to the Immune System and Inflammation. *Annals of the New York Academy of Sciences*, *917*(1), 376–386. https://doi.org/10.1111/j.1749-6632.2000.tb05402.x

Rothhammer, V., Borucki, D. M., Garcia Sanchez, M. I., Mazzola, M. A., Hemond, C. C., Regev, K., Paul, A., Kivisäkk, P., Bakshi, R., Izquierdo, G., Weiner, H. L., & Quintana, F. J. (2017). Dynamic regulation of serum aryl hydrocarbon receptor agonists in MS. *Neurology(R) Neuroimmunology & Neuroinflammation*, *4*(4), e359. https://doi.org/10.1212/NXI.0000000000000359

Ruddick, J. P., Evans, A. K., Nutt, D. J., Lightman, S. L., Rook, G. A. W., & Lowry, C. A. (2006). Tryptophan metabolism in the central nervous system: Medical implications. In *Expert Reviews in Molecular Medicine* (Vol. 8, Issue 20, pp. 1–27). Expert Rev Mol Med. https://doi.org/10.1017/S1462399406000068

Sadowska-Bartosz, I., Adamczyk-Sowa, M., Gajewska, A., & Bartosz, G. (2014). Oxidative modification of blood serum proteins in multiple sclerosis after interferon or mitoxantrone treatment. *Journal of Neuroimmunology*, *266*(1–2), 67–74. https://doi.org/10.1016/j.jneuroim.2013.11.005

Schlittler, M., Goiny, M., Agudelo, L. Z., Venckunas, T., Brazaitis, M., Skurvydas, A., Kamandulis, S., Ruas, J. L., Erhardt, S., Westerblad, H., & Andersson, D. C. (2016). Endurance exercise increases skeletal muscle kynurenine aminotransferases and plasma kynurenic acid in humans. *American Journal of Physiology-Cell Physiology*, *310*(10), C836–C840. https://doi.org/10.1152/ajpcell.00053.2016

Schröcksnadel, K., Wirleitner, B., Winkler, C., & Fuchs, D. (2006). Monitoring tryptophan metabolism in chronic immune activation. In *Clinica Chimica Acta* (Vol. 364, Issues 1–2, pp. 82–90). https://doi.org/10.1016/j.cca.2005.06.013

Shajib, M. S., & Khan, W. I. (2015). The role of serotonin and its receptors in activation of immune responses and inflammation. In *Acta Physiologica* (Vol. 213, Issue 3, pp. 561–574). Blackwell Publishing Ltd. https://doi.org/10.1111/apha.12430

Silva, N. M., Rodrigues, C. V., Santoro, M. M., Reis, L. F. L., Alvarez-Leite, J. I., & Gazzinelli, R. T. (2002). Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with Toxoplasma gondii: Induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. *Infection and Immunity*, *70*(2), 859–868. https://doi.org/10.1128/IAI.70.2.859-868.2002

Slominski, A., Semak, I., Pisarchik, A., Sweatman, T., Szczesniewski, A., & Wortsman, J. (2002). Conversion of <scp>L</scp> -tryptophan to serotonin and melatonin in human melanoma cells. *FEBS Letters*, *511*(1–3), 102–106. https://doi.org/10.1016/S0014-5793(01)03319-1

Sorgdrager, F. J. H., Naudé, P. J. W., Kema, I. P., Nollen, E. A., & De Deyn, P. P. (2019). Tryptophan metabolism in inflammaging: From biomarker to therapeutic target. In *Frontiers in Immunology* (Vol. 10, Issue OCT, p. 2565). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2019.02565

Srinivasan, V., Maestroni, G. J. M., Cardinali, D. P., Esquifino, A. I., Pandi Perumal, S. R., & Miller, S. C. (2005). Melatonin, immune function and aging. In *Immunity and Ageing* (Vol. 2, p. 17). BioMed Central. https://doi.org/10.1186/1742-4933-2-17

Steiner, L., Gold, M., Mengel, D., Dodel, R., & Bach, J.-P. (2014). The endogenous α7 nicotinic acetylcholine receptor antagonist kynurenic acid modulates amyloid-β-induced inflammation in BV-2 microglial cells. *Journal of the Neurological Sciences*, *344*(1–2), 94–99. https://doi.org/10.1016/j.jns.2014.06.032

Strasser, B., Becker, K., Fuchs, D., & Gostner, J. M. (2017). Kynurenine pathway metabolism and immune activation: Peripheral measurements in psychiatric and co-morbid conditions. *Neuropharmacology*, *112*(Pt B), 286–296. https://doi.org/10.1016/j.neuropharm.2016.02.030

Strasser, B., Geiger, D., Schauer, M., Gatterer, H., Burtscher, M., & Fuchs, D. (2016). Effects of exhaustive aerobic exercise on tryptophan-kynurenine metabolism in trained athletes. *PLoS ONE*, *11*(4), 1–10. https://doi.org/10.1371/journal.pone.0153617

Strasser, B., Geiger, D., Schauer, M., Gostner, J. M., Gatterer, H., Burtscher, M., & Fuchs, D. (2016). Probiotic supplements beneficially affect tryptophan–kynurenine metabolism and reduce the incidence of upper respiratory tract infections in trained athletes: A randomized, double-blinded, placebo-controlled trial. *Nutrients*, *8*(11), 1–15. https://doi.org/10.3390/nu8110752

Tan, L., Yu, J.-T., & Tan, L. (2012). The kynurenine pathway in neurodegenerative diseases: mechanistic and therapeutic considerations. *Journal of the Neurological Sciences*, *323*(1–2), 1–8. https://doi.org/10.1016/j.jns.2012.08.005

Tavares, R. G., Tasca, C. I., Santos, C. E. S., Alves, L. B., Porciúncula, L. O., Emanuelli, T., & Souza, D. O. (2002). Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochemistry International*, *40*(7), 621–627. https://doi.org/10.1016/S0197-0186(01)00133-4

Terness, P., Bauer, T. M., Röse, L., Dufter, C., Watzlik, A., Simon, H., & Opelz, G. (2002). Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: Mediation of suppression by tryptophan metabolites. *Journal of Experimental Medicine*, *196*(4), 447–457. https://doi.org/10.1084/jem.20020052

Tice, J. A., Kanaya, A., Hue, T., Rubin, S., Buist, D. S. M., LaCroix, A., Lacey, J. V., Cauley, J. A., Litwack,

S., Brinton, L. A., & Bauer, D. C. (2006). Risk factors for mortality in middle-aged women. *Archives of Internal Medicine*, *166*(22), 2469–2477. https://doi.org/10.1001/archinte.166.22.2469

Tiszlavicz, Z., Németh, B., Fülöp, F., Vécsei, L., Tápai, K., Ocsovszky, I., & Mándi, Y. (2011). Different inhibitory effects of kynurenic acid and a novel kynurenic acid analogue on tumour necrosis factor-α (TNF-α) production by mononuclear cells, HMGB1 production by monocytes and HNP1-3 secretion by neutrophils. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *383*(5), 447–455. https://doi.org/10.1007/s00210-011-0605-2

Tsang, B. K. T., & Macdonell, R. (2011). Multiple sclerosis: Diagnosis, management and prognosis. *Australian Family Physician*, *40*(12), 948–955.

Vécsei, L., Szalárdy, L., Fülöp, F., & Toldi, J. (2013). Kynurenines in the CNS: Recent advances and new questions. *Nature Reviews Drug Discovery*, *12*(1), 64–82. https://doi.org/10.1038/nrd3793

Viru, A., Janson, T., & Viru, M. (2005). *Effect of prolonged exercise on liver tryptophan oxygenase activity in rat*.

Walsh, N. P., Gleeson, M., Shephard, R. J., Jeffrey, M. G., Woods, A., Bishop, N. C., Fleshner, M., Green, C., Pedersen, K., Hoffman-goetz, L., Rogers, C. J., Northoff, H., Abbasi, A., & Simon, P. (2011). Position Statement. Part one: Immune function and exercise. *Exercise Immunology Review*, *17*, 6–63.

Wang, J., Simonavicius, N., Wu, X., Swaminath, G., Reagan, J., Tian, H., & Ling, L. (2006). Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *Journal of Biological Chemistry*, *281*(31), 22021–22028. https://doi.org/10.1074/jbc.M603503200

Wang, Q., Liu, D., Song, P., & Zou, M. H. (2015). Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation. In *Frontiers in Bioscience - Landmark* (Vol. 20, Issue 7, pp. 1116–1143). Frontiers in Bioscience. https://doi.org/10.2741/4363

Weinhold, M., Shimabukuro-Vornhagen, A., Franke, A., Theurich, S., Wahl, P., Hallek, M., Schmidt, A., Schinköthe, T., Mester, J., Von Bergwelt-Baildon, M., & Bloch, W. (2016). Physical exercise modulates the homeostasis of human regulatory T cells. *Journal of Allergy and Clinical Immunology*, *137*(5), 1607-1610.e8. https://doi.org/10.1016/j.jaci.2015.10.035

Wirthgen, E., Hoeflich, A., Rebl, A., & Günther, J. (2018). Kynurenic Acid: The Janus-faced role of an immunomodulatory tryptophan metabolite and its link to pathological conditions. In *Frontiers in Immunology* (Vol. 8, Issue JAN). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2017.01957

Wirthgen, E., Tuchscherer, M., Otten, W., Domanska, G., Wollenhaupt, K., Tuchscherer, A., & Kanitz, E. (2014). Activation of indoleamine 2,3-dioxygenase by LPS in a porcine model. *Innate Immunity*, *20*(1), 30–39. https://doi.org/10.1177/1753425913481252

Wolf, A. M., Wolf, D., Rumpold, H., Moschen, A. R., Kaser, A., Obrist, P., Fuchs, D., Brandacher, G., Winkler, C., Geboes, K., Rutgeerts, P., & Tilg, H. (2004). Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease. *Clinical Immunology*, *113*(1), 47–55. https://doi.org/10.1016/j.clim.2004.05.004

Zimmer, P., Stritt, C., Bloch, W., Schmidt, F. P., Hübner, S. T., Binnebößel, S., Schenk, A., & Oberste, M. (2016). The effects of different aerobic exercise intensities on serum serotonin concentrations and their association with Stroop task performance: a randomized controlled trial. *European Journal of Applied Physiology*, *116*(10), 2025–2034.

https://doi.org/10.1007/s00421-016-3456-1

8. Appendix

Publication 1:

Physiology and Behavior: 2018 194:583-587

Acute and chronic effects of exercise on the Kynurenine Pathway in humans – A brief review and future perspectives

Alan J Metcalfe#, Christina Koliamitra#, Wilhelm Bloch and Philipp Zimmer

#Author's shared first authorship

Tryptophan (TRP) is an essential amino acid. Metabolites of TRP have been identified as important mediators in immune regulation and function of the central nervous system. Inflammation strongly stimulates to the breakdown of TRP into Kynurenine (KYN), representing the initial step of the KYN pathway. Recently, exercise interventions have been able to demonstrate a modification of the KYN pathway plausibly by altering inflammation. However, modifications differ between acute and chronic exercise interventions. As such, this review examines the current studies that have investigated the effect of an acute (single bout) or chronic (training) exercise intervention on levels of TRP and KYN in both healthy and diseased populations.

Publication 2:

Journal of Sports Science and Medicine 2019 18: 669-673

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

Christina Koliamitra, Florian Javelle, Niklas Joisten, Alexander Shimabukuro-Vornhagen, Wilhelm Bloch, Alexander Schenk and Philipp Zimmer

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 (Treg). Here, we hypothesize that acute exercise activates the kynurenine pathway and physical fitness is associated with proportions of circulating anti-inflammatory Treg 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 (VO2max) 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` VO2max was strongly correlated with Treg 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 Treg levels and VO2max 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 Treg differentiation.

Publikation 3:

Journal of Neuroimmunology 2018 314:101-105

Persons with secondary progressive and relapsing remitting multiple sclerosis reveal different responses of tryptophan metabolism to acute endurance exercise and training

Jens Bansi#, Christina Koliamitra#, Wilhelm Bloch, Niklas Joisten, Alexander Schenk, Matthew Watson, Jan Kool, Dawn Langdon, Ulrik Dalgas, Jürg Kesselring, Philipp Zimmer

#Authors share first authorship

Disturbances in Tryptophan metabolism play a crucial role in MS. Exercise is suspected to counteract the progress of MS and its side effects. Current research suggests alterations of Tryptophan metabolism in healthy individuals in response to exercise. We investigated the influence of acute aerobic exercise and training on Tryptophan metabolism in 57 in persons with RRMS (n=33) and SPMS (n=24) MS. Serotonin increased after training, whereas the kynurenine pathway was only activated in persons with RRMS. Further research is warranted to investigate whether these changes are associated with clinical measures (e.g. depressions and immune function).

Personal Information

Last name:	**Koliamitra**
First name:	**Christina**
Date of birth:	**30 October 1985**
Place of birth:	**Volos, Greece**
Nationality:	**Greek, German**

Studies

Currently: **PhD Candidate**- **Scientific assistant at German Sport University of Cologne (DSHS)**, Institute for Cardiovascular Research and Sports Medicine

2012: Internship in German Sport University of Cologne (DSHS): " Influence of blood storage on the red blood cell-nitric oxide synthase phosphorylation sites" under the supervision of Prof. Dr. Wilhelm Bloch, Head Professor in Molecular and Cellular Sports Medicine department of German Sport University of Cologne

2012: Graduation from the School of Biology (Aristotle University of Thessaloniki) in the orientation of Molecular Biology, Genetics and Biotechnology

2010: Award scholarship recipient for the undergraduate mentoring program on the Annual Meeting of the American Society for Molecular Biology and Evolution (SMBE), Halifax, Nova Scotia, Canada

2009: Undergraduate studies in Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada

2009: Internship in Iakentro Fertility Clinics, Thessaloniki, Greece under the supervision of Prof. Dr. Nikolaos Prapas, Professor of Obstetrics- Gynaecology and Prof. Dr. Konstantinos Triantafylldis, Professor of Molecular Biology, Aristotle University of Thessaloniki, Greece

2008: Dissertation on "DNA barcoding in Greek freshwater fish: the cases of Doirani and Volvi lakes" under the supervision of Dr. Triantafyllidis Alexandros, Associate Professor in Population Genetics and Dr. Bobori Dimitra, Laboratory of Zoology. School of biology, Aristotle University of Thessaloniki, Greece

2007: Pre-diploma project on “Expression analysis of cDNA of bovine fetal growth plate and cartilage by EST sequencing” under the supervision of Prof. Dr. Erwin R. Schmidt, Institut für Molekulargenetik, Johannes Gutenberg-Universität Mainz, Germany

2006: Semester studies in Molecular Genetics, Department of Molecular Genetics, Johannes Gutenberg University, Mainz, Germany

2005: Admission in School of Biology in the Aristotle University of Thessaloniki

2004: Graduation from 8th Lyceum of Volos, Greece

Awards - scholarships

2018: Internal Research Funding (HIFF) in German Sport University of Cologne

2018: Career Support for Young female scientists scholarship (German Sport University of Cologne) for the American College of Sports Medicine Meeting in Minneapolis, Minnesota, USA

2017: Career Support for Young female scientists scholarship (German Sport University of Cologne) for the American College of Sports Medicine Meeting in Denver, Colorado, USA

2016: Career Support for Young female scientists scholarship (German Sport University of Cologne) for the American College of Sports Medicine Meeting in Boston, Massachusetts, USA

2012: European Erasmus Mundus Scholarship for Internship in the German Sport University of Cologne, Germany

2010: American Society for Molecular Biology and Evolution Undergraduate Award for studies in Dalhousie University in Halifax, Nova Scotia, Canada

2006: European Erasmus Scholarship for the Institute for Molecular Genetics, Johannes Gutenberg University of Mainz, Germany

Scientific Publications

Cellular immune response to acute exercise: Comparison of endurance and resistance exercise.

Schlagheck ML, Walzik D, Joisten N, **Koliamitra C**, Hardt L, Metcalfe AJ, Wahl P, Bloch W, Schenk A, Zimmer P

Eur J Haematol. July 2020. doi: 10.1111/ejh.13412

Exercise and the Kynurenine pathway: Current state of knowledge and results from a randomized cross-over study comparing acute effects of endurance and resistance training.

Joisten N, Kumerhoff F, **Koliamitra C**, Schenk A, Walzik D, Hardt L, Knoop A, Thevis M, Kiesl D, Metcalfe AJ, Bloch W, Zimmer P

Exerc Immunol Rev 2020;26:24-42

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

Koliamitra C, Javelle F, Joisten N, ShimabukuroVornhagen A, Bloch W, Schenk A, Zimmer P

J Sports Sci Med 2019 Nov 19;18(4):669-673

Impact of acute aerobic exercise on genome-wide DNA-Methylation in natural killer cells-a pilot study

Schenk A, Koliamitra C, Bauer CJ, Schier R, Schweiger MR, Bloch W, Zimmer P

Genes 2019 May 19;10(5):380. doi: 10.3390/genes10050380

Invidually tailored whole- body vibration training to reduce symptoms of chemotherapy-induced peripheral neuropathy: study protocol of a randomized controlled trial-VANISH

Streckmann F, Hess V, Bloch W, Décard BF, Ritzmann R, Lehmann HC, Balke M, **Koliamitra C**, Oschwald V, Elter T, Zahner L, Donath L, Roth R, Faude O

BMJ 2019 Apr 24;9(4):e024467 doi: 10.1136/bmjopen-2018-024467

Acute exercise increases the expression of KIR2DS4 by promoter demethylation in NK cells

Schenk A, Pulverer W, **Koliamitra C**, Bauer CJ, Ilic S, Heer R, Schier R, Schick R, Böttiger BW, Gerhäuser C, Bloch W, Zimmer P

Int J Sports Med 2019 Jan;40(1):62-70.doi: 10.1055/a-0741-7001

Acute and chronic effects of exercise on the kynurenine pathway in humans - A brief review and future perspectives

Metcalfe AJ, **Koliamitra C**, Javelle F, Bloch W, Zimmer P

Physiol Behav 2018 Oct 1; 194:583-587.doi: 10.1016/j.physbeh.2018.07.015.

Persons with secondary progressive and relapsing remitting multiple sclerosis reveal different responses of tryptophan metabolism to acute endurance exercise and training

Bansi J, **Koliamitra C**, Bloch W, Joisten N, Schenk A, Watson M, Kool J, Langdon D, Dalgas U, Kesselring J, Zimmer P

J Neuroimmunol 2018 Jan 15;314:101-105 doi: 10.1016/j.jneuroim.2017.12.001.

The preventive effect of sensorimotor- and vibration exercises on the onset of Oxaliplatin- or vinca-alkaloid induced peripheral neuropathies- STOP

Streckmann F, Balke M, Lehmann HC, Rustler V, **Koliamitra C**, Elter T, Hallek M, Leitzmann M, Steinmetz T, Heinen P, Baumann FT, Bloch W

BMC Cancer 2018 Jan 10;18 (1):62. doi: 10. 1186/s12885-017-3866-4.

Impact of training volume and intensity on RBC-NOS/NO pathway and endurance capacity.

Koliamitra C, Holtkamp B, Zimmer P, Bloch W, Grau M

Biorheology. July 2017. doi: 10.3233/BIR-16121

Acute exercise induced changes of gene-specific DNA methylation in natural killer cells.

Koliamitra C, Bloch W, Schenk A, Pulverer W, Zimmer P

Med Sci Sports Exerc. May 2017; 49 (8 Suppl 1): 288. Doi:10.1249/01.mss. 0000878896.235295.8d.

Akute belastungsinduzierte Veränderungen der genspezifischen DNA-Methylierung in natürlichen Killerzellen.

Koliamitra C, Bloch W, Schenk A, Pulverer W, Zimmer P

47. Deutscher Sportärztekongress, Frankfurt, Germany, September 2016.

Deutsche Zeitschrift für Sportmedizin 2016; 65(7-8): 180.

Chronic Training Improves RBC Deformability And Oxygen Uptake Through Increase Of Rbc-nos Activation

Koliamitra C, Holtkamp B, Zimmer P, Bloch W, Grau M.

Med Sci Sports Exerc. May 2016; 48 (5 Suppl 1): 298. Doi:10.1249/01.mss. 0000485896.83295.3d.

Chronische Auswirkung des Trainings auf die Verformbarkeit der Erythrozyten.

Koliamitra C, Holtkamp B, Bloch W, Grau M

46. Deutscher Sportärztekongress, Frankfurt, Germany, September 2015.

Deutsche Zeitschrift für Sportmedizin 2015; 65(7-8): 190.

Exercise-induced Natural Killer Cell Activation is driven by Epigenetic Modifications.

Zimmer P, Bloch W, Schenk A, Zopf EM, Hildebrandt U, Streckmann F, Beulertz J,

Koliamitra C, Schollmayer F, Baumann F. Int. J Sports Med, June 2015

Int J Sports Med 2015; DOI: 10.1055/s-0034-1398531 (IF: 2.27).

Changes of epigenetic modifications in natural killer cells of breast cancer patients in follow-up care by enhanced physical activity.

Schenk A, Baumann FT, Koliamitra C, Bloch W, Schollmayer F, Beulertz J, Zimmer P.

31. Deutscher Krebskongress, Berlin, Germany, Januar 2014.

Oncology Research and Treatment 2014; 37(suppl.1): 12.

Eine Verminderung der erythrozytären Verformbarkeit während der Blutlagerung ist mit einer Reduktion der erythrozytären Stickstoffmonoxid-Synthase Aktivierung assoziiert.

Grau M, Friederichs S, Krehan C, Koliamitra C, Suhr F, Bloch W.

33. Jahrestagung, Deutsche Gesellschaft für Klinische Mikrozirkulation und Hämorheologie Villingen-Schwenningen, Germany, November 2014.

Impact of exercise on pro inflammatory cytokine levels and epigenetic modulations of tumor-competitive lymphocytes in Non-Hodgkin-Lymphoma patients-randomized controlled trial.

Zimmer P, Baumann FT, Bloch W, Schenk A, Koliamitra C, Jensen P, Mierau A, Hülsdünker T, Reinart N, Hallek M, Elter T. Eur. J Haematol. December 2014.

Eur J Haematol 2014; 93(6): 527-532 (IF: 2,414)

Optimierung von Ausdauertrainingsprogrammen für Fitness Studios.

Koliamitra C, Zimmer P, Schenk A, Baumann F, Bloch W.

45. Deutscher Sportärztekongress, Frankfurt, Germany, September 2014.

Deutsche Zeitschrift für Sportmedizin 2014; 65(7-8): 215

Decrease in red blood cell deformability is associated with a reduction in RBC-NOS activation during storage.

Grau M, Friederichs P, Krehan S, Koliamitra C, Suhr F, Bloch W. Clinical Hemorheol. Microcirculation Journal, June 2014

Clin Hemorheol Microcirc 2014; Doi: 10.3233/CH-141850 (IF: 2,215)

Epigenetische Modifikationen in natürlichen Killer-Zellen (NK)- Einfluss von Alter und Sport.

Zimmer P, Schenk A, Koliamitra C, Jensen P, Baumann F, Hallek M, Bloch W, Elter T.

44. Deutscher Sportärztekongress, Frankfurt, Germany, September 2013.

Deutsche Zeitschrift für Sportmedizin 2013; 64(7-8): 215

DNA barcoding analysis of fish species diversity in four north Greek lakes.

Triantafyllidis A, Bobori D, Koliamitra C, Gbandi E, Mpanti M, Petriki O, Karaiskou N. Mitochondrial DNA Journal, January 2011.

Mitochondrial DANN 22,37-42. (IF: 1,705)

Work Experience

Since 01/2017: Research Associate at Institute of Cardiology and Sports Medicine, Department of Molecular and Cellular Sports Medicine, German Sport University of Cologne

2012- 2014: Research Assistant at Institute of Cardiology and Sports Medicine, Department of Molecular and Cellular Sports Medicine, German Sport University of Cologne