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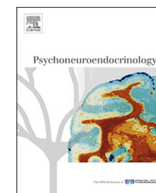

Psychosocial stress and inflammation driving tryptophan breakdown in children and adolescents: A cross-sectional analysis of two cohorts

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ABSTRACT

Background: Tryptophan breakdown is an important mechanism in several diseases e.g. inflammation and stress-induced inflammation have been associated with the development of depression via enhanced tryptophan breakdown. Depression is a major public health problem which commonly starts during adolescence, thus identifying underlying mechanisms during early life is crucial in prevention. The aim of this work was to verify whether independent and interacting associations of psychosocial stress and inflammation on tryptophan breakdown already exist in children and adolescents as a vulnerable age group.
Methods: Two cross-sectional population-based samples of children/adolescents (8–18 y) were available: 315 from the European HELENA study and 164 from the Belgian ChiBS study. In fasting serum samples, tryptophan, kynurenine, kynurenic acid, C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)-α, interferon (IFN)-ɣ, soluble vascular adhesion molecule 1 (sVCAM1) and soluble intercellular adhesion molecule 1 (sICAM1) were measured. Psychological stress was measured by stress reports (subjective) and cortisol (objective – awakening salivary cortisol or hair cortisol). Linear regressions with stress or inflammation as predictor were adjusted for age, sex, body mass index, puberty, socio-economic status and country.
Results: In both cohorts, inflammation as measured by higher levels of CRP, sVCAM1 and sICAM1 was associated with kynurenine/tryptophan ratio and thus enhanced tryptophan breakdown (beta: 0.145–0.429). Psychological stress was only associated with tryptophan breakdown in the presence of higher inflammatory levels (TNF-α in both populations).
Conclusions: Inflammatory levels were replicable key in enhancing tryptophan breakdown along the kynurenine pathway, even at young age and in a non-clinical sample. The stress-inflammation interaction indicated that only the stress exposures inducing higher inflammatory levels (or in an already existing inflammatory status) were associated with more tryptophan breakdown. This data further contributes to our understanding of pathways to disease development, and may help identifying those more likely to develop stress or inflammation-related illnesses.

1. Introduction

The tryptophan-kynurenine pathway which catabolizes 95% of tryptophan, has been linked to several diseases like psychological disorders, impaired cognition and cardiometabolic diseases (O'Farrell and Harkin, 2017; Oxenkrug, 2010a). In this tryptophan-to-kynurenine breakdown (Fig. 1), two enzymes have a leading role: indoleamine 2,3-dioxygenase (IDO1) and tryptophan 2,3-dioxygenase (TDO) (Oxenkrug, 2010b). IDO1 activity is mainly induced by pro-inflammatory molecules such as interferon (IFN)-ɣ, IFN-α and tumor necrosis factor (TNF)-α as IDO1 is widely expressed on immune cells like dendritic cells, macrophages, microglia and eosinophils (Takikawa et al., 1999). TDO is

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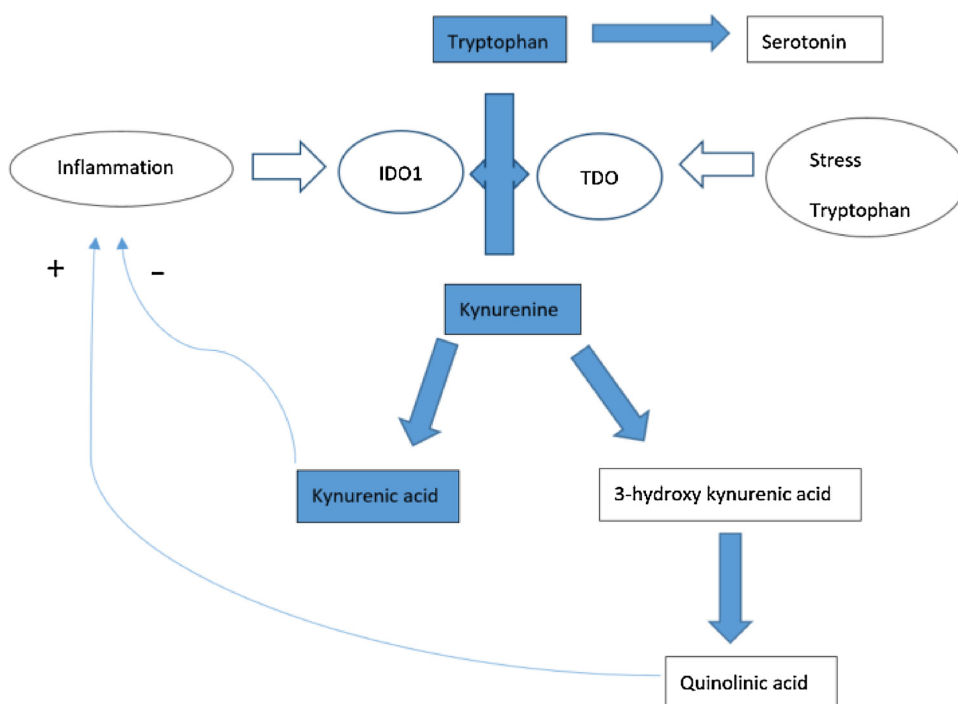

Fig. 1. The tryptophan breakdown pathway. TDO = tryptophan 2,3-dioxygenase, IDO = indoleamine 2,3-dioxygenase. Metabolites in the blue boxes were measured in the current study. Interesting, some metabolites of kynurenine like kynurenic acid are anti-inflammatory and neuroprotective, while others like quinolinic acid are pro-inflammatory and neurotoxic.

involved in the negative feedback i.e. high tryptophan will stimulate tryptophan breakdown via TDO activation, but also stress, mainly via the stress hormone cortisol, can induce TDO activity (Gibney et al., 2014). The IDO1 and TDO stimulated conversion of tryptophan to kynurenine will also lead to diminished serotonin production as tryptophan is a precursor of serotonin (Oxenkrug, 2010b). The resulting kynurenine can largely be metabolised via two pathways (Oxenkrug, 2010b; Schwarcz et al., 2012) (Fig. 1): (1) via the formation of kynurenic acid as a neuroprotective and anti-inflammatory metabolite or (2) via the predominantly neurotoxic and pro-inflammatory quinolinic acid branch because of *N*-methyl-D-aspartate receptor stimulation, lipid peroxidation and disruption of the blood-brain barrier (although also functional metabolites like NAD arise in this branch). Consequently, the kynurenine/tryptophan ratio reflects the first step of tryptophan breakdown by TDO or IDO, while the kynurenic acid/kynurenine ratio can reflect a next step away from the pro-inflammatory tryptophan metabolites.

It has now become apparent that this tryptophan-to-kynurenine breakdown contributes to the development of age-associated neuroendocrine disorders (hypertension, dyslipidaemia, type 2 diabetes, obesity, vascular cognitive impairment and some hormone-related cancers) and mood disorders (O'Farrell and Harkin, 2017; Oxenkrug, 2010a). Partially this is because of apoptotic, neurotoxic, and pro-oxidative effects of certain tryptophan metabolites like quinolinic acid; partially this is because of diminished serotonin production which is linked to mood, sleep, sexual behaviour, cognition and appetite (Hulsken et al., 2013; O'Farrell and Harkin, 2017; Oxenkrug, 2010a).

Because of IDO and TDO activity, inflammation and stress may be two pathological situations affecting tryptophan breakdown and can converge to have further health deteriorating results via this mutual pathophysiological process, especially when an optimal supply of tryptophan and its metabolites to the central nervous system is desirable. Of particular interest is the increasing amount of evidence that inflammation and chronic stress might stimulate each other via interaction between cytokines and cortisol (Hansel et al., 2010; Leonard, 2005). Nevertheless, only a few studies exist on the role of inflammation (cytokines and related molecules like acute phase proteins and cell-adhesion molecules) and stress in the tryptophan breakdown using an overall population sample (Deac et al., 2016; Elovainio et al., 2012; Pertovaara et al., 2007) and none in children. Research has often focused on patients with depression showing higher tryptophan breakdown (Maes et al., 1993; Myint et al., 2013), but not in everyone (Gabbay et al., 2010). Concerning inflammation, IFN-ɣ is known as the strongest inducer of IDO1, although this association is not always evident in healthy populations (Deac et al., 2016; Fitzgerald et al., 2008). Thus, a better understanding of the role of tryptophan breakdown following psychosocial stress and inflammation during vulnerable periods like childhood and adolescence, and hence early in the disease process, is necessary.

The goal of the current paper is to examine whether the association of inflammation and stress with tryptophan metabolites could be already observed in children and adolescents. This is important as tryptophan breakdown could then potentially be used as early target for prevention. To allow replication, the independent and interacting associations of psychosocial stress (subjectively and objectively measured) and inflammation (six markers) on enhanced tryptophan breakdown was tested in two population samples of children and adolescents.

2. Methods

2.1. Study samples

Two population samples of children/adolescents have been used.

The HELENA-Cross Sectional Study was conducted in 3528 adolescents aged 12.5–17.5 from 10 European cities from 2006 to 2007. Details on sampling procedures and study design of the HELENA study have been reported elsewhere (Moreno et al., 2008). For this paper, specific inclusion criteria were the availability of serum (and thus tryptophan and inflammation data) and the stress questionnaire (which was an optional module) and the absence of an acute infection (CRP > 10 mg/l) as was the case in 315 adolescents. Included participants seemed to have a lower BMI than those with missing data (z-score 0.2 vs 0.5), but did not differ in sex or age. Only in a subsample (n = 161, from the same six countries), salivary cortisol was available; this sample did not differ in BMI or sex but was somewhat younger (mean age 14.3 vs 14.8 y, but same age range) than the other 155 included participants.

For the longitudinal ChiBS study, participants were Dutch-speaking Belgian children (Michels et al., 2012b); 242 children aged 8–16 were included in the March-May 2015 period (as a two-hour appointment at the local sports park). Fasting serum levels were analysed on a detailed set of inflammatory and tryptophan metabolism parameters. In addition, stress was measured by hair cortisol analysis and questionnaires (stressors and emotions). None of children had a reported diagnosis of Cushing/Addison disease, auto-immune disease, acute infection or clinical depression. Nobody took oral anti-inflammatory drugs or selective serotonin reuptake inhibitors. One child using oral corticosteroids, two children with a CRP > 10 mg/l (indicative for acute infection) and three children with fever in the last 3 days were excluded. This paper included only 164 participants with complete info on questionnaire data, inflammatory markers and tryptophan-to-kynurenine breakdown. Included participants did not differ in age, BMI or sex compared to excluded ones. Only in a subsample ($n = 81$), valid hair cortisol data was available; this sample did not differ in age or BMI but included more girls (59% versus 21%) due to often too short hair in boys.

The studies were conducted according to the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital (EC/2006/086, EC/2009/688). A written informed consent was obtained from the parents and a verbal assent from the children/adolescents.

2.2. Stress measures

Stress arises when the demands of a situation exceed an individual's ability to cope and resolve the problem, resulting in emotional and behavioral disturbances (McCance et al., 2006). Via questionnaires, negative events (environmental level) and/or emotions (psychological level) are aspects that reflect stress exposure. On biological level, cortisol is the most often used biomarker and can be measured in several matrices like blood, saliva and hair (Vanaelst et al., 2012a). In HELENA, a questionnaire on negative events and salivary cortisol were used. In ChiBS, a questionnaire reflecting negative events, a questionnaire reflecting negative emotions and cortisol in hair were used.

2.2.1. Negative events in HELENA ($n = 315$)

Adolescents completed the 56-item Adolescent Stress Questionnaire (De Vriendt et al., 2011b) divided in 10 component scales: stress of home life, school performance, school attendance, romantic relationships, peer pressure, teacher interaction, future uncertainty, school/leisure conflict, financial pressure and emerging adult responsibility. Respondents were asked to indicate on a 5-point Likert scale (1 = "not at all stressful (or is irrelevant to me)", 2 = "a little stressful", 3 = "moderately stressful", 4 = "quite stressful", 5 = "very stressful") how stressful these items had been to them during the past year. A stress summary score was obtained by adding the individual scores of all 56 items.

2.2.2. Salivary cortisol in HELENA ($n = 161$)

In HELENA, baseline (without stimulation) wake-up salivary free cortisol was collected immediately after awakening. To account for intra-individual variability, awakening samples from seven consecutive days were collected (the same week as all other measures) and the mean was used. Participants got an oral explanation on the sampling and a detailed instruction sheet was provided. Saliva was collected with Salivettes® (Sarstedt, Germany), allowing stable samples at room temperature for a minimum of one week. The Salivettes® were centrifuged at 2000*g* for 10 min, and the filtrates were stored at −20 °C. Salivary cortisol was measured using a modification of an unextracted radioimmunoassay method (Diasorin) for serum cortisol (routine lab Ghent University Hospital). Briefly, 200 µl saliva was pipetted into the coated tube and incubated with ^{125}I cortisol for 45 min at 37 °C. The modified cortisol assay had a 0.5–30 µg/l measuring range. Only those adolescents who had at least three saliva samples collected between 6 a.m. and 8 a.m. were included.

2.2.3. Negative events in ChiBS ($n = 164$)

The Coddington Life Events Scale for Children (CLES-C) is a validated and well-established 36-item questionnaire (test-retest $r = 0.69$, parent-child agreement ICC = 0.45) (Coddington, 1999). By child self-report, it assesses the prevalence, frequency and timing of stressful life events relevant for this age group during the last year. By measuring significant life events in terms of Life Change Units depending on timing, frequency and severity, the questionnaire can provide insight into recent events that may affect the child's health.

2.2.4. Negative emotions score in ChiBS ($n = 164$)

Since a young childhood sample is concerned, a short and easy-to-understand questionnaire on emotions was chosen. Feelings of anger, anxiety and sadness could be rated on a 0 (not at all) to 10 (very strong) Likert-scale. The negative emotions score (sum of three negative emotions) showed a Spearman correlation of $r = 0.48$ ($p < 0.001$) with the negative affect score of the validated PANAS-C questionnaire (Laurent et al., 1999) (tested in a subsample i.e. participant in our 2013 study wave that were at least 9 y old).

2.2.5. Hair cortisol in ChiBS ($n = 81$)

Hair cortisol has recently been established as a reliable correlate of chronic stress exposure (Wester and van Rossum, 2015). Only the most proximal 3 cm of the hair strands from the vertex posterior were analysed. Since hair grows approximately 1 cm each month, this 3 cm reflects the exposure during the last 3 months. Extraction and liquid chromatography coupled with tandem mass spectrometry was performed at the Laboratory for Hormonology, Ghent University Hospital. To start, 15 mg of hair was milled in a Retch Ballmill MM200. After addition of 2 ml Sorenson buffer and 20 µl of Cortisol-d4 internal standard, hair samples were incubated overnight at 42 °C. Then, 1.8 ml of buffer 100 µl NaOH (1 M) was added and mixed. After extraction with 2.5 ml diethylether and mixing for 3 min, samples were frozen and decanted with subsequent drying of the collected supernatant. The dried supernatant was then reconstituted in a final solution of 125 µl methanol of which 100 µl was injected for liquid chromatography. Cortisol was acquired from Sigma Aldrich (Saint Louis, USA), d4-cortisol from CDN Isotopes (Quebec, Canada). All standards and internal standards were dissolved in methanol. An AB Sciex 5500 triple-quadrupole mass spectrometer (AB Sciex; Toronto, Canada) was used, coupled with an APCI probe on the Turbo-V source. The liquid chromatography system consisted of a Shimadzu system using a C8 security guard column (5 um,4 × 2 mm) and a C8 Luna analytical column (3um, 50 × 3 mm) (Phenomenex; Torrance, USA). Measurements were performed by the tandem mass spectrometer running in multiple reaction monitoring mode by using transitions *m/z* 363/121/97 for cortisol and d4-cortisol on *m/z* 367/121/97. A declustering potential of 100 V and a collision energy 32 eV was used for all the analytes. Data processing was performed through MultiQuant version 2.0.2. Inter-assay CV for cortisol was 10.8% with a limit-of-quantification of 1.6 pg/mg hair.

2.3. Biomarkers of inflammation

Six inflammatory parameters were analysed in fasting serum after storage at −80 °C: Acute-phase protein C-reactive protein (CRP), IL-6, TNF-α, IFN-ɣ soluble vascular adhesion molecule 1 (sVCAM1) and soluble intercellular adhesion molecule 1 (sICAM1). Participants with fever during the last 24 h were excluded and nobody used nonsteroidal anti-inflammatory drugs. For ChiBS, samples were analysed in duplicate by enzyme-linked immunosorbent assay (ELISA) using electrochemiluminescent multiplex kits (VPlex by Meso Scale Discovery, Maryland, USA) with a Sector 2400 analyser at the APC microbiome institute at the University College Cork in Ireland (intra-assay CV 2–4%,

inter-assay CV 5–11%). For HELENA, CRP was measured in serum by immunoturbidimetry (AU2700 biochemistry analyzer; Olympus, Rungis, France) while IL-6, TNF-α, IFN-ɣ, sVCAM1 and sICAM1 were determined using the High Sensitivity Human Cytokine MILLIPLEXTM MAP kit (Millipore Corp., Billerica, MA, USA) and collected by flow cytometry (Luminex–100 v.2.3, Luminex Corporation, Austin, TX, USA). Including or excluding samples with values below the detection limit (< 5% of samples) did not change the results.

2.4. Tryptophan breakdown

For both ChiBS and HELENA serum samples, tryptophan, kynurenine and kynurenic acid were determined at the APC microbiome institute at University College Cork in Ireland. Briefly, serum samples were spiked with internal standard (3-Nitro-L-tyrosine) prior to being deproteinised by the addition of 20 µl of 4 M perchloric acid to 200 µl of sample. Samples were centrifuged at 21,000*g* on a Hettich Mikro 22R centrifuge (AGB, Dublin, Ireland) for 20 min at 4 °C and 100 µl of supernatant transferred to a HPLC vial for analysis on the HPLC system (UV and FLD detection). All samples were injected onto a reversed phase Luna 3 µm C18 (2) 150 × 2 mm column (Phenomenex), which was protected by KrudKatcher disposable pre-column filters (Phenomenex) and SecurityGuard cartridges (Phenomenex). The mobile phase consisted of 50 mM acetic acid and 100 mM zinc acetate with 3% (*v*/*v*) acetonitrile and was filtered through Millipore 0.45-µm HV Durapore membrane filters (AGB) and vacuum degassed prior to use. Compounds were eluted isocritically over a 30-min runtime at a flow rate of 0.3 ml/min after a 20-µl injection. The column was maintained at a temperature of 30 °C, and samples/standards were kept at 8 °C in the cooled autoinjector prior to injection. The fluorescent detector was set at an excitation wavelength of 254 nm and an emission wavelength of 404 nm. The UV detector was set to 330 nm. L-tryptophan, kynurenine and kynurenic acid were identified by their characteristic retention times as determined by standard injections which were run at regular intervals during the sample analysis. Internal standard peak height ratios were measured and compared with standard injections, and results were expressed as nanogram per millilitre of serum. Apart from the three single metabolites (blue boxes in Fig. 1), we used the kynurenine/tryptophan ratio as marker of enhanced tryptophan breakdown by TDO or IDO and the kynurenic acid/kynurenine ratio as marker of the next breakdown step away from the predominantly pro-inflammatory and neurotoxic quinolinic acid pathway.

2.5. Possible confounders

Pubertal status (stages I–V) was assessed by a doctor using the Tanner and Whitehouse criteria (Tanner and Whitehouse, 1976). Body mass index (BMI) was calculated by dividing measured weight by height squared (kg/m^2) and the BMI z-score was calculated (Cole et al., 2000) while overweight was classified following the International Obesity Task Force. To represent socio-economic status, parental education level was assessed by questionnaire according to the International Standard Classification of Education. Diet was not included in the regression since neither tryptophan intake nor protein intake was related to any of the tryptophan serum variables.

2.6. Statistical analyses

Statistical analyses were performed using SPSS 22 and all p-values < 0.05 were considered significant. Spearman correlations were performed for continuous variables. Linear regression analyses tested the association of stress and inflammation with tryptophan status (tryptophan, kynurenine, kynurenic acid, kynurenine/tryptophan and kynurenic acid/kynurenine) after adjustment for age, sex, BMI, puberty, socio-economic status (and in the HELENA population also country). First, a multivariate linear regression with all six inflammatory parameters together was run to see the overall inflammation association (without multiple testing). Second, separate models for stress and inflammation as predictor of interest were tested. Third, inflammation and stress were put together in one model to detect independent associations. Standardized regression coefficients were reported. When the regression residuals were not normally distributed, outcome variables were transformed by calculating the logarithm, which was the case for kynurenic acid and the kynurenic acid/kynurenine ratio. No significant sex*stress or sex*inflammation interaction was found.

Finally, the moderation of inflammation in the stress-tryptophan relation was tested. This was only performed for the theoretically most relevant tryptophan parameter i.e. the kynurenine/tryptophan ratio by adding the interaction term "stress*inflammation" as predictor of kynurenine/tryptophan, after centring the predictors. In the case of a significant interaction, the stress – kynurenine/tryptophan relation was tested for 3 representative groups: those at the mean, at 1 SD below the mean and 1 SD above the mean of the inflammatory parameter.

Sensitivity analysis: Running all analyses on the subsample that had also cortisol values (n = 81 for ChiBS, n = 161 for HELENA) did not change the main results, there was only one significant finding in the HELENA sample that became borderline significant (p = 0.052, beta = 0.140 for the association between sICAM1 and kynurenine/tryptophan).

3. Results

3.1. Descriptive data

Descriptive data for both population samples is given in Table 1. All children in the ChiBS sample came from Belgium; the age ranged from 8 to 16 years; whereas HELENA is a European representative sample with children coming from: 28.8% Belgium, 24.9% Sweden, 17.2% Spain, 13.1% Hungary, 9.5% Austria, 6.5% Greece; the age ranged from 12.5 to 17.5y. The two population samples differed in almost all parameters of interest and were thus rather complementary: the ChiBS population had a younger age group, higher proportion of females, higher socio-economic status, but lower adiposity, kynurenine/tryptophan and inflammatory levels with the exception of IFN-ɣ, sICAM1 and TNF-α. OF course, it should be considered that age might be a reason for different serum levels. A correlation matrix can be found in Supplementary material.

3.2. Association of inflammation with tryptophan breakdown

A multivariate linear regression showed an overall significant association between inflammation parameters and kynurenine/tryptophan ratio (ChiBS: p < 0.001, partial eta squared = 0.228; HELENA: p = 0.056, partial eta squared = 0.110). Table 2 presents the linear regression results for inflammation as predictor of tryptophan pathway parameters in both studies. In both samples, higher CRP, sVCAM1 and sICAM1 were positively associated with a higher kynurenine/tryptophan ratio. We also observed positive associations of IFN-ɣ and TNF-α with this kynurenine/tryptophan ratio in the ChiBS and HELENA sample, respectively. In line with this ratio, higher kynurenine (with CRP and IFN-ɣ) and lower tryptophan levels (with CRP and sVCAM1) were related to inflammation in ChiBS. Kynurenic acid was not related to the inflammatory parameters. After Bonferroni correction (p = 0.004 i.e. 0.05/12 since six inflammatory parameters were tested in two populations), all 4 findings related to kynurenine/tryptophan in the ChiBS sample (CRP, IFN-ɣ, sVCAM1, sICAM1) remained significant. Supplementary Table 2 represents the same results but with inflammatory parameters scaled per interquartile range (IQR) to get an idea of effect sizes. The maximum effect size on kynurenine/tryptophan was by CRP in the ChiBS population: one IQR change in CRP was associated with 0.006 units increase in kynurenine/tryptophan, which is half of an IQR increase (see Table 1), thus a rather large effect size.

Table 1
Descriptive data of the two population samples.

	HELENA (n = 315) Median [p25; p75] or %	ChiBS (n = 164) median [p25; p75] or %	p-value: HELENA vs ChiBS
sex (% male)	42.7%	54.8%	< 0.001
age (years)	14.5 [13.6; 15.3]	12.6 [11.8.13.8]	< 0.001
BMI (z-score)	0.2 [−0.5; 0.9]	−0.4 [−0.9; 0.2]	< 0.001
overweight (%)	14.6%	3.6%	< 0.001
SES (% high)	61.7%	83.1%	< 0.001
puberty (% pubertal)	94.6%	88.2%	0.030
serum values			
L-Tryptophan (ng/ml)	11,555 [10,185; 13,257]	11,913 [10,620; 13,640]	0.291
L-Kynurenine (ng/ml)	573 [485; 695]	677 [566; 772]	< 0.001
kynurenine/tryptophan	0.049 [0.043; 0.056]	0.056 [0.051; 0.062]	< 0.001
kynurenic acid (ng/ml)	3.61 [1.77; 4.87]	3.07 [0; 5.56]	0.129
kynurenic acid/kynurenine	0.006 [0.003; 0.009]	0.004 [0; 0.009]	0.010
CRP (mg/l)	0.556 [0.221; 1.281]	0.267 [0.108; 0.833]	< 0.001
IL-6 (pg/ml)	10.02 [4.44; 25.56]	1.07 [0.64; 1.87]	< 0.001
TNF-α (pg/ml)	5.74 [4.02; 8.11]	5.87 [4.50; 7.47]	0.059
IFN-γ (pg/ml)	0.90 [0.12; 6.97]	4.32 [2.5; 6.7]	< 0.001
sVCAM1 (mg/l)	1.196 [0.972; 1.513]	0.657 [0.579; 0.787]	< 0.001
sICAM1 (mg/l)	0.122 [0.098; 0.161]	0.498 [0.415; 0.571]	< 0.001
stress variables			
Cortisol: saliva (HELENA, n = 161) or hair (ChiBS, n = 81))	23.06 nmol/l [18.81; 27.67]	3.77 pg/mg [2.78; 5.18]	
events: ASQ (HELENA) or CLES (ChiBS)	123 [87; 157]	60 [17; 122]	
negative emotions (0–30)	/	7 [5; 10]	

BMI = body mass index; SES = socio-economic status by parental education; CRP = C-reactive protein; IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; sVCAM = soluble vascular cell adhesion molecule; sICAM = soluble intercellular adhesion molecule; ASQ = adult stress questionnaire; CLES = Coddington Life Events Scale.

3.3. The role of stress in tryptophan breakdown

Since we hypothesize that inflammation observed in this group could be due to stress, we added stress variables to the above mentioned regression, but no changes in significant findings were detected (data not shown). Indeed, Table 3 shows that none of the stress parameters was significantly associated with the tryptophan pathway parameters. In Supplementary Fig. 1, scatterplots clearly show that stress is only related to some inflammatory markers (sVCAM1 and sICAM1) and only in the ChiBS population. Finally, when checking their interactive effect, significant moderation between reported stress (events or emotions) and inflammation was found in their association with the kynurenine/tryptophan ratio. Replicable in both populations (ChiBS and HELENA), a positive association between stress reports and kynurenine/tryptophan was found only in case of high TNF-α levels (Fig. 2). For IFN-γ ($p < 0.001$; R^2change = 0.09), sVCAM1 ($p = 0.007$; R^2change = 0.04) or sICAM ($p < 0.001$, R^2change = 0.06), this was only the case in the ChiBS population.

4. Discussion

Replicable in two independent population samples of children/adolescents, inflammation (high CRP, sVCAM1 and sICAM1) was confirmed as a key positive predictor of the kynurenine/tryptophan ratio (Table 2). The resulting lowered tryptophan during this vulnerable age-period might be a pathway to higher morbidity (e.g. psychological disorders, impaired cognition and cardiometabolic disorders) including ones that typically develop during adolescence such as major depressive disorders. Although stress reports and cortisol levels were not associated with tryptophan metabolism (Table 3), significant stress-

Table 2
Linear regression of inflammation parameters with tryptophan metabolites as outcome for the ChiBS (n = 164) and HELENA (n = 315) population.

		tryptophan		Kynurenine		kynurenine/tryptophan		kynurenic acid		kynurenic acid/kunyrenine	
		HELENA	ChiBS	HELENA	ChiBS	HELENA	ChiBS	HELENA	ChiBS	HELENA	ChiBS
CRP	beta	−0.088	**−0.170**	0.059	**0.226**	**0.145**	**0.429**	0.117	0.083	−0.056	0.046
	P	0.358	**0.032**	0.481	**0.004**	**0.035**	**< 0.001**	0.143	0.292	0.478	0.562
TNF-α	beta	0.010	−0.108	0.091	−0.044	**0.112**	0.089	−0.002	0.040	−0.036	0.054
	P	0.859	0.178	0.091	0.586	**0.040**	0.267	0.971	0.623	0.465	0.504
IL-6	beta	−0.001	0.026	−0.001	0.068	−0.006	0.070	0.002	0.009	−0.048	0.009
	p	0.999	0.747	0.988	0.404	0.914	0.392	0.699	0.908	0.382	0.909
IFN-γ	beta	0.021	−0.110	0.063	**0.201**	0.073	**0.390**	−0.023	−0.009	−0.031	−0.024
	p	0.694	0.173	0.227	**0.021**	0.177	**< 0.001**	0.646	0.908	0.541	0.764
sICAM1	beta	0.005	−0.117	0.132	0.148	**0.169**	**0.292**	0.093	0.123	0.049	0.130
	p	0.951	0.157	0.063	0.074	**0.021**	**< 0.001**	0.177	0.140	0.429	0.117
sVCAM1	beta	−0.085	**−0.216**	0.065	0.143	**0.192**	**0.340**	−0.059	−0.022	−0.032	0.045
	p	0.295	**0.006**	0.358	0.070	**0.012**	**< 0.001**	0.419	0.783	0.621	0.570

CRP = C-reactive protein; IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; sVCAM = soluble vascular cell adhesion molecule; sICAM = soluble intercellular adhesion molecule. Standardized regression coefficients are shown from linear regression adjusted for age, sex, parental education, BMI, puberty (and for the HELENA population also country). Bold values = $p < 0.05$.

Table 3
Linear regression of psychological stress with tryptophan metabolites as outcome for the ChiBS and HELENA population.

		tryptophan	kynurenine	kynurenine/tryptophan	kynurenic acid	kynurenic acid/kunyrenine
HELENA						
salivary awakening cortisol (n = 161)	beta	0.001	0.067	0.092	−0.043	−0.048
	p	0.985	0.368	0.218	0.578	0.514
ASQ (n = 315)	beta	0.023	−0.023	−0.074	−0.001	0.011
	p	0.677	0.661	0.171	0.998	0.845
ChiBS						
hair cortisol (n = 81)	beta	−0.119	−0.139	−0.065	−0.089	0.108
	p	0.258	0.176	0.524	0.393	0.308
CLES (n = 164)	beta	0.090	−0.016	−0.065	0.078	0.014
	p	0.266	0.847	0.422	0.335	0.862
negative emotions score (n = 164)	beta	−0.113	−0.119	0.009	−0.084	−0.071
	p	0.174	0.154	0.910	0.316	0.396

ASQ = adult stress questionnaire; CLES = Coddington Life Events Scale. Standardized regression coefficients are shown from linear regression adjusted for age, sex, parental education, BMI, puberty (and for the HELENA population also country).

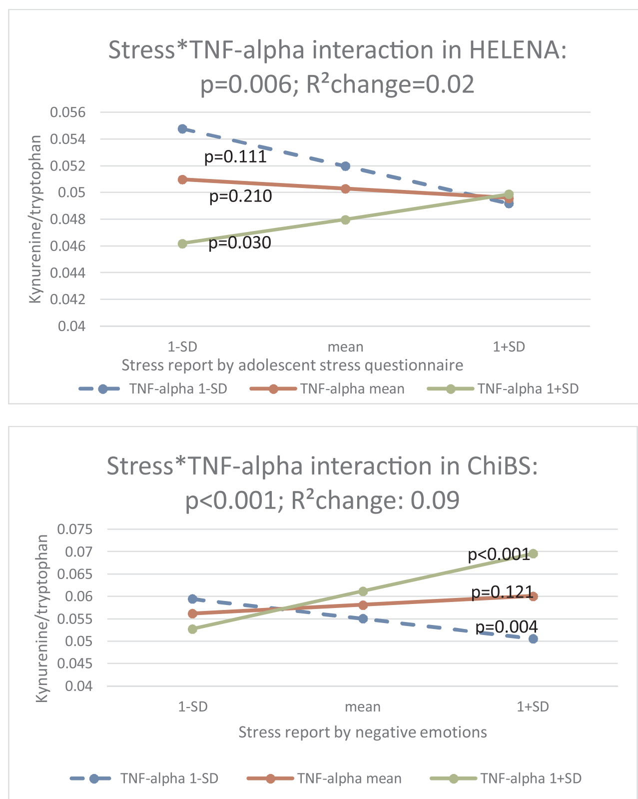

Fig. 2. Stress-inflammation interaction replicable in both the ChiBS (n = 166) and HELENA (n = 316) population.
TNF-α = tumor necrosis factor α.
The moderation of inflammation in the association between stress and kynurenine/tryptophan was tested by adding the interaction term "stress*inflammation" as predictor. Only the significant interactions are shown. The stress – kynurenine/tryptophan relation is shown for 3 representative groups: those at the mean, at 1 SD below the mean and 1 SD above the mean of the inflammatory parameter.

inflammation interaction was detected (Fig. 2) since stress reports were associated with higher kynurenine/tryptophan in case of higher inflammatory levels (replicable for TNF-α in both samples); reflecting a stress-associated inflammation guiding of tryptophan breakdown.

4.1. Association of inflammation with tryptophan breakdown

We first showed that inflammatory parameters were associated with higher tryptophan breakdown towards kynurenine. A multivariate linear regression showed an overall significant association between inflammatory parameters and kynurenine/tryptophan with moderate effect size.

In order to better understand mechanisms, we investigated which particular inflammatory parameter induced tryptophan breakdown. CRP, sICAM1 and sVCAM1 significantly increased tryptophan breakdown via IDO1 enzyme activity in both study populations. CRP as general acute-phase-protein seemed to be the strongest parameter (beta = 0.429); this has also been observed in Finnish healthy adults (Pertovaara et al., 2007) but not in Irish healthy adults (Deac et al., 2016). We were the first to show an association of the atherosclerotic and inflammation-related cell-adhesion molecules sICAM1 and sVCAM1 with tryptophan breakdown. These cell adhesion molecules had not been tested yet by others in relation to kynurenine/tryptophan, although increased kynurenine/tryptophan ratio has been associated with cardiovascular risk (Pertovaara et al., 2007). IFN-ɣ and TNF-α were also associated with tryptophan breakdown but only in one of our two study populations. This role of IFN-ɣ and TNF-α is in agreement with other studies (Deac et al., 2016; Myint et al., 2013; Wolowczuk et al., 2012), although IFN-ɣ is not related to kynurenine/tryptophan in all studies (Deac et al., 2016; Fitzgerald et al., 2008).

In our sample, no significant associations with kynurenic acid or its ratio with kynurenine were found. Similar to our study, no association between these inflammatory parameters and kynurenic acid or kynurenine/kynurenic acid ratio is present in a sample of healthy adults (Deac et al., 2016).

4.2. The role of stress in tryptophan breakdown

Next, we hypothesized that the inflammation-induction of tryptophan breakdown towards kynurenine would be particularly important in those with higher levels of stress as stress might stimulate TDO (Salter and Pogson, 1985) via cortisol or inflammatory molecules. We were particularly interested in individuals high in both stress and inflammation, since research shows that in patients with chronic stress (e.g. major depressive disorder) increased tryptophan breakdown occurs in the presence of inflammation (Maes et al., 1993; Myint et al., 2013). To test this hypothesis, we analysed whether stress moderated the association between inflammation and tryptophan metabolites. Neither subjective stress as measured by questionnaires nor objective stress as measured by cortisol levels were associated with tryptophan breakdown alone. However, there was a significant interaction between stress and TNF-α in tryptophan breakdown in both population samples showing a moderation effect of stress. This moderation might be translated as: only stress exposures that induce higher inflammatory levels (or in the presence of an already existing inflammatory status) were associated with more tryptophan breakdown. In this perspective, subjective stress was not related to higher inflammatory markers in the

whole population (no overall significant positive correlations between stress and inflammation observed in our population) but only in some individuals. Reasons for increased stress-induced inflammation in some individuals is unknown but may involve among others the duration/level of stress e.g. childhood maltreatment and poverty; or individual vulnerability like genetics and epigenetic changes (Oxenkrug, 2010a; Slavich and Irwin, 2014). This was not investigated in this study.

To our knowledge, we are the first to report on the stress-inflammation and tryptophan breakdown association in a population sample of children/adolescents. Some reports exist on the direct role of stress: stress induction in mice increases kynurenine/tryptophan ratio (Fuertig et al., 2016; Kiank et al., 2010; Liu et al., 2015), while depressive symptoms are related to higher kynurenine/tryptophan in one study (Elovainio et al., 2012) but not in another (Fitzgerald et al., 2008). Nevertheless, human studies have not yet tested the stress-inflammation interaction in tryptophan breakdown. Confirming our moderation effect, a cross-talk between inflammation and tryptophan breakdown has been demonstrated in animal models with chronic stress. For example, stress-induced IDO expression in mice can be blocked by anti-TNF-α treatment (Kiank et al., 2010; Liu et al., 2015). Theoretically, stress-inflammation interaction is suggested by IDO decreasing TDO expression (Guillemin et al., 2007) and by mutual cortisol-inflammation stimulation (Turck et al., 2005; Zunszain et al., 2011). Thus, the combination of chronic inflammation and stress might change tryptophan metabolism (Zunszain et al., 2011).

This tryptophan breakdown has many health implications. Through formation of kynurenine toxic metabolites, tryptophan is broken down away from the production of the neuroprotective serotonin (Leonard, 2005). In addition, kynurenine shifted towards production of neurotoxic metabolites is implicated in neurodegeneration and immune tolerance (Guillemin et al., 2007). Together these changes could lead to brain damage, finally evolving towards mental health problems including depression (Myint et al., 2007; Myint et al., 2012). Therefore, in children with high levels of stress and inflammation, increased tryptophan breakdown towards toxic kynurenine metabolites may be a pathway by which stress leads to diseases which often start during adolescence such as major depression. Targeting inflammation and/or tryptophan breakdown in children with high levels of stress may be a strategy to prevent the development of depression in vulnerable children.

4.3. Strengths and limitations

A major advantage with existing literature was the use of two population-based samples of children/adolescents (range 8–18 y), allowing replicable testing whether the expected associations occur already early in life to indicate pathways for prevention. Current literature is mainly based on animal and clinical samples and population results are scarce, especially in such a young group. The children in these two cohorts had both subjective and objective measures of stress and inflammatory markers measured. The HELENA dataset is transnational and therefore results may be more easily and robustly generalized.

Yet, the current study has its limitations. A major disadvantage was the cross-sectional design not allowing assumptions about cause-effect directionality. Indeed, bidirectionality might be possible e.g. higher IDO-activity can induce depressive symptoms or enhanced stress vulnerability (Elovainio et al., 2012; Leonard, 2005). In addition, tryptophan depletion has been shown to influence salivary cortisol levels (Vielhaber et al., 2005). A second limitation is the different methodology to measure stress within the two studies: different event questionnaires were used, the ChiBS study also included an emotion report, and the two studies used a different matrix for cortisol analysis. Of note, our stress reports have previously been validated against cortisol levels (Michels et al., 2012a). Herein, salivary cortisol sampling in the HELENA dataset is less sensitive or representative for long-term cortisol exposure than hair cortisol in the ChiBS dataset, especially since only the awakening cortisol and not the diurnal patterns were available (Golden et al., 2011), but hair-saliva inter-correlations have been reported in literature (Vanaelst et al., 2012b). Also, the stress questionnaire in the HELENA study shows less than optimal repeatability and criterion validity (De Vriendt et al., 2011a). A third and important limitation is that only kynurenine and kynurenic acid but no other metabolites were determined like quinolinic acid to measure health-deteriorating pathways of tryptophan breakdown or serotonin to measure the other important tryptophan pathway. This decision was taken due to additional financial costs and due to interpretation uncertainty of these metabolites' blood concentration: transport of serotonin and quinolinic acid through the blood-brain barrier is questionable (Fukui et al., 1991; Nakatani et al., 2008) and blood serotonin might reflect rather peripheral processes (e.g. serotonin production in the gastro-intestinal tract). Nevertheless, the kynurenine/tryptophan ratio might be influenced by this downstream kynurenine breakdown towards quinolinic acid, instead of purely reflecting IDO1 activity as is hypothesized in this manuscript. Thus further research on these downstream metabolites will better frame the health effects.

5. Conclusion

This study demonstrated by replication in two independent population samples that already during childhood/adolescence inflammation leads to higher tryptophan breakdown and thus to further morbidity in this vulnerable age-group. Moreover, the significant stress-inflammation interaction indicated that only stress exposures inducing higher inflammatory levels (or in the presence of an already existing inflammatory status) were associated with increased tryptophan breakdown. This might enable the identification of an at-risk population with higher stress and inflammation vulnerability that are then at risk for health-deteriorating effects of tryptophan breakdown.

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Conflict of interest

None. The financing bodies were not involved in data analyses/interpretation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.psyneuen.2018.05.013.

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