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A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study

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Summary

Circulating neopterin and kynurenine/tryptophan ratio (KTR) increase during inflammation and serve as markers of cellular immune activation, but data are sparse on other determinants of these markers and metabolites of the kynurenine pathway. We measured neopterin, tryptophan, kynurenine, anthranilic acid, kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid and xanthurenic acid in plasma in two age groups, 45–46 years ($n = 3723$) and 70–72 years ($n = 3329$). Differences across categories of the potential determinants, including age, gender, renal function, body mass index (BMI), smoking and physical activity, were tested by Mann–Whitney *U*-test and multiple linear regression including age group, gender, renal function and lifestyle factors. In this multivariate model, neopterin, KTR and most kynurenines were 20–30% higher in the older group, whereas tryptophan was 7% lower. Men had 6–19% higher concentrations of tryptophan and most kynurenines than women of the same age. Compared to the fourth age-specific estimated glomerular filtration rate (eGFR) quartile, the first quartile was associated with higher concentrations of neopterin (25%) and KTR (24%) and 18–36% higher concentrations of kynurenines, except 3-hydroxyanthranilic acid. Additionally, KTR, tryptophan and all kynurenines, except anthranilic acid, were 2–8% higher in overweight and 3–17% higher in obese, than in normal-weight individuals. Heavy smokers had 4–14% lower levels of tryptophan and most kynurenines than non-smokers. Age and renal function were the strongest determinants of plasma neopterin, KTR and most kynurenines. These findings are relevant for the design and interpretation of studies investigating the role of plasma neopterin, KTR and kynurenines in chronic diseases.

Keywords: indoleamine 2, 3-dioxygenase, inflammation, kynurenine to tryptophan ratio, neopterin

Introduction

Inflammation plays a central role in the pathogenesis of many chronic diseases, such as cardiovascular disease and cancer [1]. In increased cellular immune activation interferon (IFN)-γ stimulates the production of neopterin by macrophages and additionally increases the conversion of tryptophan (Trp) to kynurenine (Kyn) by up-regulating the enzyme indoleamine 2,3-dioxygenase (IDO) [2,3]. Trp can also be converted to Kyn by tryptophan 2,3-dioxygenase (TDO), an enzyme with lower affinity for Trp, and responsible for metabolizing Trp that exceeds the requirements for protein and serotonin synthesis [3]. Therefore, the plasma kynurenine/tryptophan ratio (KTR) is influenced by the activities of both IDO and TDO, while plasma neopterin reflects only IFN-γ activity [4]. More than 90% of Trp is metabolized through the kynurenine pathway to compounds collectively named kynurenines [3]. After the rate-limiting conversion of Trp to Kyn, Kyn is metabolized further to anthranilic acid (AA), kynurenic acid (KA) or 3-hydroxykynurenine (HK), which is converted to either 3-hydroxyanthranilic acid (HAA) or xanthurenic acid (XA) (Fig. 1).

Both neopterin [5] and KTR [6] have been found to be associated with chronic diseases. A number of kynurenines, such as Kyn, HK, HAA and KA, have been reported to play a

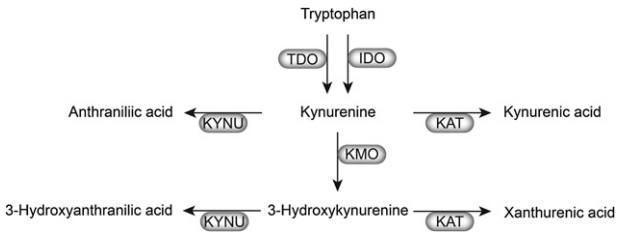

Fig. 1. The kynurenine pathway. Tryptophan is converted to kynurenine either by indoleamine 2,3-dioxygenase (IDO) or by tryptophan 2,3-dioxygenase (TDO). Kynurenine is metabolized further to anthranilic acid by kynureninase (KYNU), kynurenic acid by kynurenine aminotransferase (KAT) or 3-hydroxykynurenine by kynurenine mono-oxygenase (KMO). 3-Hydroxykynurenine is converted in turn to either 3-hydroxyanthranilic acid by KYNU or xanthurenic acid by KAT.

role in immune regulation [7]. Additionally, several kynurenines have been associated with autoimmune diseases [6], infection [6], cancer [6], neuroendocrine disorders [8] and metabolic syndrome [8]. In studies examining the relation of these markers and metabolites to disease outcomes, it is important to be aware of their potential determinants in order to account for possible confounding. Data on variations in neopterin, KTR and kynurenines according to age [9–13], gender [12–15], renal function [16–18], overweight/obesity [19–23] and smoking [9,15] are sparse or fragmentary, while data on the potential effects of physical activity are lacking. A thorough investigation of the importance of such factors is motivated by the considerable renal clearance of kynurenines [17], the increased IFN-γ activity accompanying obesity [4], the anti-inflammatory effect of physical activity [24] and the known immunomodulatory effects of smoking [25]. We therefore investigated age, gender, renal function, body mass index (BMI), smoking and physical activity as determinants of neopterin, KTR and kynurenines in a large community-based cohort of middle-aged and elderly men and women.

Materials and methods

Study population

The source population consisted of subjects born in 1925–27 or 1950–51 and residing in the city of Bergen (Norway) or the neighbouring suburban municipalities ($n = 9187$) who participated in the Hordaland Health Study (HUSK) during 1997–99. The overall attendance rate was 77%, providing a sample of 7052 participants in the age groups 46–47 years (2062 women and 1661 men) and 70–72 years of age (1860 women and 1469 men). HUSK is a collaboration between the National Health Screening Service, University of Bergen, University of Oslo and local health services in the Bergen area. The study protocol was approved by the Western Norway Regional Committee for Medical Research Ethics and by the Norwegian Data Inspectorate. All participants gave written informed consent.

Blood samples and biochemical analyses

Non-fasting blood samples were collected into tubes containing ethylenediamine tetraacetic acid (EDTA) and stored at 4–5°C within 15–30 min. Samples were centrifuged within a maximum of 3 h, and EDTA plasma was stored at −80°C until analysis. Neopterin, Trp and six kynurenines (Kyn, AA, KA, HK, HAA and XA), as well as cotinine, an established marker of recent nicotine exposure [26], were measured using a high-throughput liquid chromatography tandem mass spectrometry (LC-MS/MS) assay [27]. KTR was calculated by dividing the plasma concentration of Kyn by the concentration of Trp and subsequently multiplying by 1000. Serum creatinine was measured by including it and its deuterated internal standard (d3-creatinine) in an established high-performance liquid chromatography (HPLC)-MS/MS assay [28] using the ion pairs 114/44·2 and 117/47·2, respectively, and was used for calculating the estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration [29] equation. All biochemical analyses were performed in the laboratory of Bevital AS (http://www.bevital.no). Within-day coefficients of variance (CVs) for neopterin, Trp and kynurenines were 1·8–9·5% and between-day CVs were 5·0–16·9% [27].

Information on lifestyle

Height and weight were measured following standard protocols used by the National Health Screening Service, and BMI was calculated as weight/height^2 (kg/m^2). Three categories were defined according to BMI using the World Health Organization's cut-off points: normal weight (BMI < 25 kg/m^2), overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2) [30]. A self-administered questionnaire was used to collect information on smoking status (current, former or never). In addition, we measured plasma cotinine to define never smokers (plasma cotinine ≤ 85 nmol/l), former smokers (plasma cotinine ≤ 85 nmol/l and self-reported previous smoking), moderate smokers (cotinine between 86 and 1199 nmol/l) and heavy smokers (cotinine ≥ 1200 nmol/l). The self-administrated questionnaire also included questions on physical activity during the last year, with light physical activity defined as activity without sweating or becoming out of breath, and heavy physical activity defined as activity with sweating or becoming out of breath. Participants reporting less than 1 h of heavy physical activity per week were classified as having a low level of physical activity. Those reporting 1 h or more of heavy physical activity per week were classified as having a moderate level of physical activity.

Table 1. Characteristics of participants in the Hordaland Health Study stratified by age and gender.

	46–47 years		70–72 years	
	Women ($n = 2062$)	Men ($n = 1661$)	Women ($n = 1860$)	Men ($n = 1469$)
Renal function				
Creatinine (μmol//l)	72 (60–88)	86 (72–104)*	76 (60–100)†	91 (72–123)*†
eGFR (ml/min/1·73 m^2)‡	86 (68–105)	92 (73–106)*	69 (49–88)†	72 (51–89)*†
Body mass index				
BMI (kg/m^2)	24·1 (19·9–32·6)	25·9 (21·4–31·8)*	26·0 (19·6–34·1)†	25·8 (21·0–31·5)
< 25	1230 (60)	621 (37)*	768 (41)†	565 (39)*
25–30	629 (31)	846 (51)*	751 (41)†	763 (52)*
> 30	201 (10)	192 (12)	335 (18)†	138 (9)*
Smoking				
Never smoker	802 (40)	550 (34)*	1101 (61)†	340 (24)*†
Former smoker	466 (23)	456 (28)*	404 (22)†	812 (56)*†
Current smoker	749 (37)	622 (38)	303 (17)†	293 (20)†
Cotinine (nmol/l)	2·5 (0–1801)	4·4 (0–2071)*	0·5 (0–1290)†	0·9 (0–1694)*†
Physical activity				
Low	625 (30)	364 (22)*	1051 (57)†	623 (42)*†
Moderate	1437 (70)	1297 (78)*	809 (43)†	846 (58)*†

Values are median (5th–95th percentile) or *n* (%). Due to missing data, the number of observations was lower for BMI ($n = 7039$) and smoking ($n = 6988$). *$P < 0·01$ for difference between genders within age group, by Mann–Whitney *U*-test or χ^2 test. †$P < 0·01$ for difference between age groups within gender, by Mann–Whitney *U*-test or χ^2 test. ‡eGFR was calculated using the chronic kidney disease-Epi formula (CKD-Epi). eGFR: estimated glomerular filtration rate; BMI: body mass index.

Data analysis

Subjects' characteristics are presented as medians (5th, 95th percentiles) for continuous variables, and as counts (proportions) for discrete variables. Age-specific probability density plots show the distributions of neopterin, KTR, Trp and kynurenines. Partial Spearman's correlations adjusted for age group and gender were used to investigate correlations between neopterin, KTR, Trp and kynurenines. Comparisons of plasma concentrations between subgroups of determinants and a selected subgroup for each determinant used as a reference group were made using the Mann–Whitney *U*-test for continuous variables, χ^2 test for categorical variables and test for trend across quartiles of eGFR. Associations of determinants with neopterin, KTR and kynurenines were investigated using multiple linear regression models with log-transformed outcome variables (natural logarithm). The multivariate model included age group, gender, renal function, BMI categories, physical activity and smoking. The back-transformed regression coefficients estimate the proportional difference in geometric means of each category compared to the reference group and are presented as proportional (%) difference relative to the reference group. Renal function was included in the model as age-specific quartiles of eGFR, with the highest quartile as reference. A test for trend was used across quartiles of eGFR and BMI categories. As the effects of smoking on the immune system may be multi-faceted [25], we estimated differences rather than a test for trend using analysis of variance (ANOVA). All analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), except the probability density plots that were produced using R (version 2.14.1 for Windows) [31], package sm [32]. Statistical tests were two-tailed, with a *P*-value < 0·01 considered significant.

Results

Population characteristics

The study population consisted of 3723 participants aged 46–47 years (middle-aged) and 3329 participants aged 70–72 years (elderly). In the elderly group eGFR was lower than in the middle-aged group. Approximately 40% of the middle-aged women and 60% of the middle-aged men and elderly participants of both genders were overweight or obese. Smoking and moderate physical activity were more prevalent among the middle-aged than among the elderly subjects (Table 1).

Intercorrelations of neopterin, KTR and kynurenines

Neopterin and KTR were correlated strongly ($r = 0·47$). Both neopterin and KTR were associated moderately positively with AA ($r = 0·22$ for both), KA ($r = 0·20$ and $r = 0·27$, respectively) and HK ($r = 0·31$ and $r = 0·33$, respectively), but not with the downstream catabolites of HK, HAA ($r = 0·08$ and $r = 0·05$, respectively) or XA (no significant correlation and $r = -0·07$, respectively). Among the kynurenines, HAA and XA showed the strongest positive correlations with Trp ($r = 0·39$, for both), whereas AA, KA and HK were only associated weakly with Trp ($r < 0·15$). All

Table 2. Spearman's correlations of neopterin, KTR, Trp and kynurenines in the Hordaland Health Study.

	Neopterin	KTR	Trp	Kyn	AA	KA	HK	HAA
KTR	0·47							
Trp	–0·18	–0·49						
Kyn	0·39	0·67	0·24					
AA	0·22	0·22	0·09	0·34				
KA	0·20	0·27	0·12	0·42	0·31			
HK	0·31	0·33	0·14	0·50	0·21	0·43		
HAA	0·08	0·05	0·39	0·38	0·25	0·42	0·47	
XA	0·01*	–0·07	0·39	0·24	0·18	0·55	0·44	0·54

Correlations are adjusted for age group and gender. All associations without a footnote mark are significant at $P < 0{\cdot}001$; *$P = 0{\cdot}57$. KTR: kynurenine/tryptophan ratio; AA: anthranilic acid; HAA: 3-hydroxyanthranilic acid; HK: 3-hydroxykynurenine; KA: kynurenic acid; Kyn: kynurenine; Trp: tryptophan; XA: xanthurenic acid.

kynurenines were correlated positively with Kyn ($r = 0{\cdot}24$–$0{\cdot}50$) (Table 2). All correlations mentioned were statistically significant ($P < 0{\cdot}001$).

Age and gender

In both age groups, the distributions of plasma neopterin, KTR and kynurenines were right-skewed, while the distribution of Trp was close to normal (Fig. 2). Details on the age- and gender-specific distributions of neopterin, KTR, Trp and kynurenines are presented in online Supplementary Table S1. Median concentrations of neopterin, KTR, Kyn, AA, KA and HK were 21–32% higher in elderly *versus* middle-aged individuals ($P < 0{\cdot}01$) (Table 3). The differences between age groups remained significant after adjustment for gender, renal function, BMI, physical activity and smoking ($P < 2 \times 10^{-16}$). After adjustment, older compared to younger subjects had 27% [99% confidence interval (CI): 25–29%] higher neopterin, 30% (27–32%) higher KTR and 20–24% higher Kyn, AA, KA and HK; all associations were highly significant ($P < 2 \times 10^{-16}$) (Table 4).

Median plasma neopterin concentrations were 6% lower in men than in women in the middle-aged group, but there were no gender differences for neopterin in the elderly. In neither age group did KTR differ between genders. However, median concentrations of Trp, Kyn, KA, HAA and XA were 10–18% higher in men than in women of the same age ($P < 0{\cdot}01$ for all differences) (Table 3). After adjustment for age group, renal function, BMI, physical activity and smoking, men had 10–19% higher concentrations of Trp, Kyn, KA, HAA and XA compared to women; all associations mentioned were highly significant ($P < 2 \times 10^{-16}$) (Table 4).

Renal function

Plasma concentrations of neopterin, KTR and all kynurenines, except HAA, decreased significantly across quartiles of eGFR in both age groups (P for trend $< 0{\cdot}001$) (Table 3). The same trends were found in the multivariate models adjusted for age group, gender, BMI, smoking and physical activity (P for trend $< 2 \times 10^{-16}$). In the multivariate model the first quartile of eGFR was associated with 25% (99% CI: 22–28%) higher concentrations of neopterin, 24% (21–27%) higher KTR and 18–36% higher concentrations of the kynurenines, except HAA, compared to the fourth quartile (Table 4).

BMI and physical activity

Neopterin did not differ across BMI categories, but KTR, Trp and all kynurenines, except AA in middle-aged

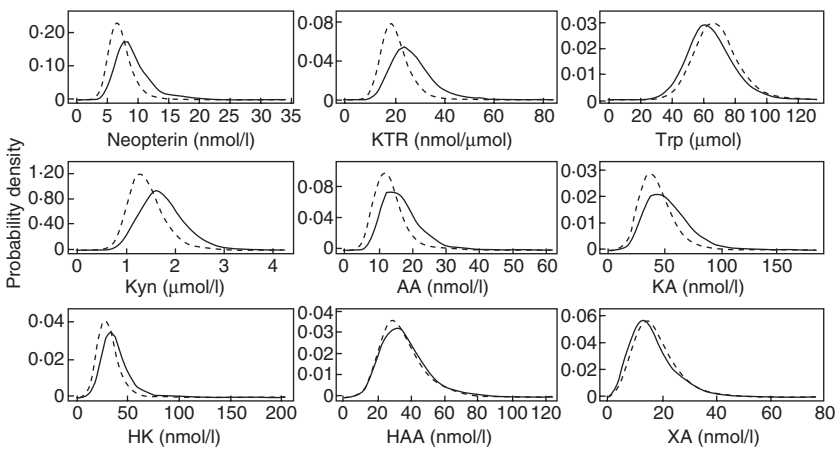

Fig. 2. Age-specific probability density plots of neopterin, KTR, Trp and kynurenines in the Hordaland Health Study. Probability density functions of neopterin, KTR and kynurenines in the middle-aged group, aged 45–46 years (dashed line) and in the elderly group, aged 70–72 years (solid line). KTR: kynurenine/tryptophan ratio; AA: anthranilic acid; HAA: 3-hydroxyanthranilic acid; HK: 3-hydroxykynurenine; KA: kynurenic acid; Kyn: kynurenine; Trp: tryptophan; XA: xanthurenic acid.

Table 3. Plasma concentrations of neopterin, KTR, Trp and kynurenines, according to gender, renal function and lifestyle factors in the Hordaland Health Study.

	Neopterin (nmol/l)	KTR (nmol/μmol)	Trp (μmol/l)	Kyn (μmol/l)	AA (nmol/l)	KA (nmol/l)	HK (nmol/l)	HAA (nmol/l)	XA (nmol/l)
Total									
Age 45–46 years ($n = 3723$)	6·9 (4·7–10·9)	20·0 (13·4–31·2)	68·9 (48·6–95·5)	1·38 (0·94–2·09)	12·9 (7·7–22·2)*	41·7 (24·1–72·9)	29·5 (17·4–50·5)	32·6 (17·8–60·2)	16·1 (7·0–33·8)
Age 70–72 years ($n = 3229$)	8·7 (5·7–15·6)*	26·3 (16·4–43·9)*	64·8 (44·4–93·3)*	1·71 (1·10–2·63)*	16·3 (9·9–28·8)*	51·1 (27·6–92·7)*	35·6 (21·0–65·0)*	34·2 (18·1–64·5)*	15·0 (6·1–32·9)*
Age 45–46 years									
Gender									
Women ($n = 2062$)	7·1 (4·8–11·3)	19·9 (13·5–31·3)	65·9 (46·4–91·5)	1·30 (0·89–2·02)	12·7 (7·6–22·1)	39·1 (22·9–68·4)	30·3 (17·6–53·5)	31·1 (17·4–59·4)	15·3 (6·4–34·0)
Men ($n = 1661$)	6·7 (4·6–10·4)*	20·1 (13·2–31·1)	72·5 (53·4–98·2)*	1·47 (1·02–2·16)*	12·9 (7·9–22·3)	45·1 (26·2–79·1)*	28·7 (17·3–46·7)*	34·6 (18·19–60·8)*	17·1 (7·9–33·6)*
eGFR (ml/min/1·73 m^2)‡									
Q1 (< 80·6)	7·5 (5·0–12·4)	21·5 (14·6–34·4)	68·5 (48·3–93·9)	1·46 (0·99–2·23)	13·8 (8·2–23·9)	45·2 (26·1–81·0)	32·1 (18·9–56·4)	32·4 (17·5–60·9)	17·0 (7·4–37·3)
Q2 (80·6–88·8)	7·1 (4·9–10·7)	20·2 (13·7–30·2)	68·3 (47·5–93·6)	1·39 (0·95–2·06)	13·1 (8·0–22·1)	43·3 (24·5–72·5)	30·0 (18·3–49·7)	32·3 (18·6–62·4)	16·0 (7·0–32·9)
Q3 (88·8–98)	6·8 (4·6–10·5)	19·7 (13·2–30·2)	69·0 (48·2–97·4)	1·36 (0·95–2·04)	12·6 (7·7–21·3)	41·6 (24·9–69·5)	29·1 (16·8–48·0)	32·9 (17·3–58·6)	16·2 (7·0–33·8)
Q4 (> 98)	6·4 (4·3–10·1)†	18·6 (12·6–28·9)†	69·9 (49·8–97·1)	1·30 (0·88–2·00)†	12·00 (7·1–20·4)†	37·5 (21·6–65·3)†	26·9 (16·7–46·4)†	33·0 (17·7–58·9)	15·2 (6·5–30·9)†
BMI (kg/m^2)									
< 25 ($n = 1998$)	6·9 (4·7–11·0)	19·6 (13·2–30·7)	67·5 (47·8–94·5)	1·33 (0·90–2·02)	12·9 (7·6–22·1)	39·9 (23·2–68·5)	28·7 (17·2–48·0)	30·6 (17·3–56·3)	15·6 (6·7–32·9)
25–30 ($n = 1187$)	6·9 (4·7–10·8)	20·1 (13·6–31·1)	70·3 (49·8–95·5)*	1·41 (0·97–2·10)*	12·8 (7·8–21·9)	43·5 (25·0–74·7)*	29·7 (17·8–51·3)*	33·7 (19·1–61·0)*	16·5 (7·5–33·6)*
> 30 ($n = 393$)	6·8 (4·8–10·8)	21·8 (14·2–32·5)*	70·4 (48·8–97·4)*	1·53 (1·01–2·29)*	12·8 (7·8–23·0)	45·8 (26·2–81·7)*	32·4 (17·6–56·8)*	38·2 (18·4–68·4)*	17·5 (6·9–36·4)*
Physical activity									
Low ($n = 989$)	6·9 (4·7–11·1)	20·1 (13·0–30·9)	68·4 (47·9–94·6)	1·34 (0·91–2·05)	12·7 (7·6–22·1)	40·2 (22·8–69·4)	29·8 (17·3–51·6)	32·3 (16·5–59·9)	16·0 (6·6–33·3)
Moderate ($n = 2735$)	6·9 (4·7–10·9)	20·0 (13·5–31·3)	69·1 (48·8–95·6)	1·39 (0·94–2·10)	12·9 (7·8–22·2)	42·2 (24·8–74·0)*	29·4 (17·5–50·0)	32·8 (18·0–60·5)	16·1 (7·1–34·0)
Smoking									
Never ($n = 1352$)	7·0 (4·8–11·0)	20·1 (13·2–31·8)	69·1 (48·5–97·0)	1·40 (0·95–2·11)	13·4 (8·4–22·8)	42·3 (25·1–75·3)	29·5 (17·6–51·9)	32·6 (18·5–62·4)	16·0 (7·0–33·7)
Former ($n = 922$)	7·0 (4·8–11·7)	20·5 (13·9–32·7)	69·8 (49·9–96·7)	1·43 (0·99–2·16)*	13·6 (8·5–23·4)	44·6 (23·9–74·0)	30·1 (17·4–52·0)	33·5 (18·0–60·0)	17·1 (7·5–36·2)*
Current ($n = 1371$)	6·7 (4·6–10·7)*	19·5 (13·3–29·8)	68·0 (47·3–94·0)	1·33 (0·91–1·99)*	11·8 (7·0–19·5)*	39·6 (23·5–66·8)*	29·2 (17·3–48·7)	32·1 (16·6–58·5)	15·6 (6·6–30·9)*
Moderate smoker ($n = 662$)	6·7 (4·6–10·6)	19·4 (13·2–30·3)	68·9 (47·9–95·3)	1·34 (0·91–2·02)	12·1 (7·3–19·9)	40·4 (23·7–68·2)	29·6 (18·0–50·4)	33·2 (18·1–60·1)	16·2 (7·4–31·9)
Heavy smoker ($n = 709$)	6·7 (4·6–10·7)	19·8 (13·6–29·4)	67·0 (46·9–93·0)*	1·32 (0·91–1·95)	11·5 (6·9–19·2)*	38·4 (23·5–66·0)	28·6 (17·1–46·9)	31·0 (15·4–56·9)*	14·8 (6·3–30·6)*
Age 70–72 years									
Gender									
Women ($n = 1860$)	8·7 (5·7–15·6)	26·3 (16·3–43·5)	62·4 (42·5–89·8)	1·65 (1·05–2·53)	15·9 (9·7–28·1)	48·1 (26·8–86·8)	35·3 (20·5–66·1)	32·7 (17·6–61·0)	14·0 (5·6–30·6)
Men ($n = 1469$)	8·6 (5·8–15·8)	26·1 (16·4–44·2)	67·9 (47·9–96·2)*	1·79 (1·20–2·47)*	16·8 (10·0–29·1)*	54·5 (29·9–98·5)*	36·2 (21·8–64·6)*	36·0 (19·3–68·3)*	16·5 (7·1–34·7)*
eGFR (ml/min/1·73 m^2)‡									
Q1 (< 62·5)	10·1 (6·8–18·6)	31·3 (18·2–52·4)	62·4 (42·7–92·9)	1·95 (1·26–2·94)	18·3 (10·9–31·8)	62·9 (33·5–121·2)	42·2 (25·6–78·1)	36·1 (19·6–68·8)	17·2 (7·1–37·4)
Q2 (62·5–70·1)	8·8 (5·9–15·6)	26·7 (16·9–42·7)	65·3 (44·2–92·8)	1·73 (1·15–2·6)	16·4 (9·9–27·5)	52·2 (29·2–89·0)	36·5 (21·9–59·4)	33·9 (18·0–64·5)	15·2 (6·1–32·6)
Q3 (70·2–78·7)	8·2 (5·8–13·5)	25·5 (16·9–37·6)	65·9 (45·9–92·8)	1·67 (1·13–2·40)	15·7 (9·7–28·9)	48·3 (29·0–83·6)	33·7 (20·5–58·7)	33·7 (18·8–63·2)	14·4 (6·7–30·6)
Q4 (> 78·7)	7·7 (5·0–12·5)†	23·3 (15·0–36·5)†	65·9 (44·3–95·2)†	1·52 (1·01–2·28)†	14·7 (9·1–26·3)†	42·3 (23·9–75·7)†	31·7 (18·5–53·7)†	33·2 (17·0–62·0)†	13·2 (5·2–28·7)†
BMI (kg/m^2)§									
< 25 ($n = 1331$)	8·7 (5·7–16·3)	25·1 (15·9–42·7)	63·4 (43·6–91·1)	1·61 (1·51–2·44)	15·9 (9·6–28·7)	46·3 (26·3–83·5)	33·7 (20·4–61·2)	32·1 (17·0–61·0)	13·7 (5·4–30·4)
25–30 ($n = 1514$)	8·5 (5·7–15·4)	26·7 (16·6–45·2)*	65·9 (45·4–94·4)*	1·75 (1·15–2·67)*	16·5 (10·0–28·8)*	53·1 (29·4–96·8)*	36·2 (22·0–64·6)*	35·0 (19·5–65·2)*	15·9 (6·7–32·9)*
> 30 ($n = 473$)	8·8 (5·7–15·2)	28·1 (17·3–43·9)*	65·5 (44·6–93·1)*	1·84 (1·17–2·77)*	16·7 (10·1–29·4)*	58·4 (30·2–103·9)*	39·7 (21·7–71·4)*	37·6 (19·7–70·9)*	16·1 (7·1–36·5)*
Physical activity									
Low ($n = 1674$)	8·7 (5·7–15·6)	26·5 (16·1–44·0)	64·3 (44·5–93·2)	1·70 (1·09–2·63)	16·3 (9·9–29·9)	49·8 (27·3–94·8)	35·4 (20·4–66·2)	33·7 (17·6–65·5)	14·4 (6·1–32·9)
Moderate ($n = 1655$)	8·6 (5·7–15·8)	26·0 (16·6–43·7)	65·2 (44·3–93·6)	1·72 (1·11–2·62)	16·3 (9·9–27·9)	51·9 (28·4–91·5)*	35·8 (21·7–62·9)	34·6 (18·8–63·8)	15·6 (6·3–32·8)*
Smoking¶									
Never ($n = 1441$)	8·6 (5·6–15·4)	25·9 (16·1–43·9)	64·6 (45·1–94·6)	1·68 (1·09–2·58)	16·2 (10·0–29·1)	49·9 (28·5–91·8)	35·2 (20·7–62·9)	34·1 (18·3–63·7)	14·9 (6·3–32·4)
Former ($n = 1216$)	8·8 (5·9–16·0)	27·0 (16·7–43·0)*	66·5 (44·9–93·9)	1·80 (1·16–2·70)*	17·2 (10·5–30·3)*	54·4 (28·6–96·3)*	36·8 (21·9–66·8)*	35·9 (19·4–68·5)*	16·0 (6·8–33·8)*
Current ($n = 596$)	8·5 (5·5–15·9)	25·6 (15·9–45·0)	62·3 (42·1–89·9)*	1·61 (1·05–2·54)*	14·2 (8·8–25·7)*	46·8 (24·7–86·4)*	34·5 (20·4–61·2)	30·8 (16·8–57·7)*	13·5 (5·2–31·3)*
Moderate smoker ($n = 337$)	8·4 (5·4–16·3)	25·7 (15·9–45·0)	63·0 (42·6–89·7)	1·63 (1·05–2·65)	14·8 (8·8–26·0)	48·2 (25·1–87·7)	34·7 (20·4–64·3)	31·6 (16·8–57·8)	13·8 (5·3–31·1)
Heavy smoker ($n = 259$)	8·6 (5·6–15·2)	25·5 (16·1–45·1)	60·3 (41·6–90·0)	1·58 (1·07–2·51)	13·8 (8·5–25·1)	45·6 (24·0–79·1)	34·4 (20·7–60·9)	29·6 (15·7–57·7)	13·2 (5·0–31·9)

Values are median (5th–95th percentile). *$P < 0·01$ for difference between groups, using the Mann–Whitney U-test. †$P < 0·01$ for trend. ‡eGFR was calculated using the chronic kidney disease-Epi formula (CKD-Epi). §All BMI groups are compared with normal-weight participants, BMI < 25 kg/m^2. ¶Current and former smokers are compared to never smokers; moderate smokers (85 nmol/l < plasma cotinine < 1200 nmol/l) are compared to heavy smokers (plasma cotinine ≥ 1200 nmol/l). KTR: kynurenine/tryptophan ratio; AA: anthranilic acid; HAA: 3-hydroxyanthranilic acid; HK: 3-hydroxykynurenine; KA: kynurenic acid; Kyn: kynurenine; Trp: tryptophan; XA: xanthurenic acid; eGFR: estimated glomerular filtration rate; BMI: body mass index.

Table 4. Adjusted proportional differences (%) of geometric means across subgroups of determinants of neopterin, KTR, Trp and kynurenines in the Hordaland Health Study obtained by multiple linear regression.

	Neopterin	KTR	Trp	Kyn	AA	KA	HK	HAA	XA
Age (years)									
Reference: 45–46	0	0	0	0	0	0	0	0	0
70–72	27 (25, 29)	30 (27, 32)	7 (8, 5)	21 (19, 23)	24 (21, 27)	20 (18, 23)	21 (19, 24)	2 (0, 5)	−9 (−12, −6)
P	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	0·02	$1{\cdot}7 \times 10^{-14}$
Gender									
Reference: female	0	0	0	0	0	0	0	0	0
Male	0 (−2, 2)	3 (1, 5)	10 (8, 11)	13 (11, 14)	6 (4, 8)	19 (16, 21)	0 (−2, 3)	10 (7, 12)	17 (14, 21)
P	0·80	$1{\cdot}3 \times 10^{-4}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$1{\cdot}3 \times 10^{-11}$	$< 2 \times 10^{-16}$	0·72	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$
Quartile of eGFR*									
Reference: Q4	0	0	0	0	0	0	0	0	0
Q3	6 (4, 9)	7 (5, 10)	0 (−2, 2)	7 (5, 10)	5 (2, 8)	13 (10, 16)	6 (3, 9)	1 (−3, 4)	10 (5, 14)
Q2	12 (9, 15)	12 (9, 15)	−1 (−2, 1)	12 (9, 14)	8 (5, 12)	19 (16, 23)	12 (9,16)	2 (−1, 6)	13 (8, 18)
Q1	25 (22, 28)	24 (21, 27)	−2 (−3, 0)	22 (20, 25)	18 (15, 22)	36 (32, 41)	26 (22, 30)	6 (2, 9)	25 (19, 30)
P for trend	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	0·01	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$8{\cdot}22 \times 10^{-06}$	$< 2 \times 10^{-16}$
BMI									
Reference: < 25 kg/m^2	0	0	0	0	0	0	0	0	0
25–30 kg/m^2	−2 (−4, 0)	3 (1, 5)	2 (0, 3)	5 (3, 6)	−1 (−3, 1)	7 (5, 9)	4 (2, 7)	8 (5, 10)	7 (4, 11)
> 30 kg/m^2	−2 (−4, 1)	9 (6, 12)	3 (1, 6)	12 (10, 15)	−1 (−4, 3)	16 (12, 20)	11 (8, 15)	17 (13, 21)	13 (7, 18)
P for trend	0·03	$2{\cdot}2 \times 10^{-14}$	$1{\cdot}1 \times 10^{-05}$	$< 2 \times 10^{-16}$	0·31	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$1{\cdot}1 \times 10^{-12}$
Physical activity									
Reference: moderate	0	0	0	0	0	0	0	0	0
Low	1 (−1, 3)	0 (−1, 2)	0 (−2, 1)	0 (−1, 2)	1 (−1, 3)	−3 (−5, −1)	0 (−3, 2)	−1 (−4, 1)	−3 (−6, 1)
P	0·33	0·51	0·67	0·70	0·18	$1{\cdot}2 \times 10^{-4}$	0·66	0·20	0·03
Smoking†									
Reference: never smoker	0	0	0	0	0	0	0	0	0
Former smoker	2 (0, 5)	3 (1, 5)	−1 (−3, 1)	2 (0, 4)	2 (0, 5)	0 (−2, 3)	3 (0, 5)	1 (−2, 4)	2 (−2, 6)
Moderate smoker	−2 (−4, 1)	0 (−3, 2)	−3 (−4, −1)	−3 (−5, −1)	−10 (−13, −7)	−4 (−7, −1)	1 (−2, 4)	−3 (−6, 1)	−3 (−8, 1)
Heavy smoker	0 (−3, 2)	2 (−1, 4)	−6 (−8, −4)	−4 (−6, −2)	−14 (−17, −11)	−7 (−10, −4)	0 (−4, 3)	−9 (−12, −5)	−11 (−15, 7)
P for difference‡	$4{\cdot}7 \times 10^{-15}$	$< 2 \times 10^{-16}$	4×10^{-4}	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$	$1{\cdot}77 \times 10^{-07}$	$1{\cdot}4 \times 10^{-10}$	$1{\cdot}1 \times 10^{-4}$

Multiple linear regression was performed on logarithmically transformed values. All variables (age group, gender, eGFR quartiles, BMI categories, physical activity group and smoking categories) were included in the model simultaneously. Results are presented as proportional differences (99% confidence interval) relative to the reference group, obtained by back-transformation of betas, $n = 6962$. *Age-specific cut-off points for quartiles of eGFR (ml/min/1·73 m^2) for age group 45–46 years: 80·6, 88·8 and 98; for age group 70–72 years: 62·5, 70·1 and 78·7; eGFR was calculated using the chronic kidney disease-Epi formula (CKD-Epi). †Moderate smokers (85 nmol/l < plasma cotinine < 1200 nmol/l), heavy smokers (plasma cotinine ≥ 1200 nmol/l). ‡Differences between the groups were tested by analysis of variance. KTR: kynurenine/tryptophan ratio; AA: anthranilic acid; HAA: 3-hydroxyanthranilic acid; HK: 3-hydroxykynurenine; KA: kynurenic acid; Kyn: kynurenine; Trp: tryptophan; XA: xanthurenic acid; eGFR: estimated glomerular filtrationrate; BMI: body mass index.

individuals, were higher in obese and overweight compared to normal-weight individuals for both age groups (Table 3). In the multivariate model, the largest differences between BMI categories were observed for HAA and decreased in magnitude in the order XA, KA, Kyn, HK, KTR and Trp, with concentrations 2–8% higher in overweight and 3–17% higher in obese than in normal-weight individuals (Table 4).

In both age groups, participants with moderate physical activity had slightly higher plasma KA concentrations compared to participants with low physical activity and, among the elderly, individuals with moderate physical activity also had higher concentrations of XA (Table 3). After multivariate adjustment, KA was 3% higher in participants with moderate compared to low physical activity ($P = 1{\cdot}2 \times 10^{-4}$), whereas the association of moderate physical activity with XA was no longer significant ($P = 0{\cdot}03$) (Table 4).

Smoking

In the middle-aged group, former smokers had lower concentrations of Kyn and XA than never smokers, whereas current smokers had lower concentrations of neopterin and all kynurenines except HK and HAA than never smokers. However, in the elderly group plasma concentrations of all kynurenines, except HK, were the highest in former smokers and the lowest in current smokers, whereas neopterin concentrations did not differ between smoking categories (Table 3). After multivariate adjustment, former smokers had 3% higher KTR and HK than never smokers. In contrast, heavy smoking was associated inversely with AA, XA, HAA, KA, Trp and Kyn, in decreasing strength of association, with 4–14% lower concentrations in heavy smokers compared to never smokers. Neopterin was not associated with smoking in the multivariate model (Table 4).

Discussion

This community-based study among 7052 individuals investigated potential determinants of plasma neopterin, KTR and a large panel of kynurenines. Higher concentrations of neopterin, KTR and most kynurenines were observed in elderly compared to middle-aged subjects, and concentrations of Trp and most kynurenines were higher in men than in women. Furthermore, renal function was associated inversely with plasma levels of neopterin, KTR and most kynurenines. Lastly, higher concentrations of KTR, Trp and most kynurenines were found in overweight/obese compared to normal-weight participants, whereas Trp and most kynurenines were lower in heavy than in never smokers.

Age and gender

The higher plasma levels of neopterin and KTR observed in the older group are in agreement with previous studies [9–12,33]. In the present study, elevated KTR in the elderly was driven mainly by markedly increased Kyn concentrations, indicating a more pronounced IDO activation in this age group. Elevated neopterin and KTR indicate increased IFN-γ activity in the older group, accompanying age-related inflammation [1]. Older age was also associated with higher concentrations of all kynurenines, except XA. Others have reported no association of age with serum Kyn [13] or KA [34]. This discrepancy may be explained by a smaller sample size ($n < 50$) in previous studies.

We observed lower neopterin in men than in women in the middle-aged group, but not in the elderly. This observation is in accordance with published results [12]. There was no difference in KTR between genders in the present study in subjects aged 45–72 years, which is in agreement with a previous study on subjects older than 50 years of age [15], but in contrast to an observation of higher KTR in men in a younger population (21–64 years) [14]. This indicates no differences in activities of IDO or TDO between genders among middle-aged and elderly people, but possibly in younger subjects, including premenopausal women. The higher concentrations of Trp and most kynurenines in men may be related to higher protein intake and/or turnover; the latter may be explained by higher muscle mass in men. The downstream effects on most kynurenines may simply reflect that Trp availability increases the flux through the kynurenine pathway, as more than 90% of Trp is metabolized through this pathway [3].

Renal function

The higher concentrations of neopterin, KTR and kynurenines in individuals with moderately reduced renal function – indicated by lower eGFR (eGFR < 98 ml/min/1·73 m^2 in the middle-aged and eGFR < 78·7 ml/min/1·73 m^2 in the elderly) – are in line with studies in patients with severe renal disease reporting increased plasma concentrations of neopterin [18], Kyn [16,17] and KA [17]. Trp was not associated with reduced renal function either in the present study or among patients with chronic kidney disease [17], in contrast to a previous report of lower Trp in patients with severe renal failure [16]. Results from an animal model support that the higher levels of Kyn in renal failure are attributed mainly to a combination of increased TDO activity and decreased kynureninase activity in the liver, and not to impaired renal excretion [16]. Conversely, the increased neopterin concentrations are attributed most probably to increased cellular immunity activation accompanying reduced renal function [18].

BMI and physical activity

Overall, the examined lifestyle factors associated with inflammation [3,22,24,25,35] were weaker determinants of circulating markers of cellular immune activation and kynurenines compared to the biological determinants.

Despite the fact that obesity is related to increased IFN-γ activity [4], BMI was not associated with neopterin in this or in a previous study [19]. In contrast, some studies indicate a positive association of BMI with neopterin [12,22,23], and inconsistencies might relate partly to the different study designs; one of the studies included mainly overweight and obese participants [22], whereas another presented only crude associations [23].

In contrast to the null findings for neopterin, we observed that overweight and obesity were associated positively with KTR and all kynurenines, except AA, which is in line with previous studies on KTR [20,21]. Thus, it is possible that kynurenines are involved in obesity and/or obesity-related conditions. Interestingly, HAA and HK can induce the formation of free radicals [36] and thereby may mediate oxidative stress associated with obesity [37]. Furthermore, XA can react with insulin and therefore may lead potentially to insulin resistance [4], a condition related strongly to obesity [37]. Finally, we observed recently that KA is a strong predictor of pre-eclampsia in obese women [38].

It has been shown that physical activity has an anti-inflammatory effect [24] and is associated with a reduction in visceral fat mass. In the present study, physical activity was not associated with neopterin, KTR or kynurenines, except for a weak inverse association between physical activity and KA. Previous studies on the short-term effect of intense exercise have reported an increase in both neopterin [39,40] and Kyn [35]. Conceivably, short-term and habitual physical activity may have different effects on IFN-γ-mediated pathways, as demonstrated previously for several inflammatory markers [24].

Smoking

In this community-based study we did not observe an association of current smoking with neopterin or KTR, as both Trp and Kyn were decreased slightly in moderate smokers and decreased further among heavy smokers; therefore, KTR was not changed in any of the groups. We also found a similar inverse association between smoking and all other kynurenines, except HK. In a recent study in cardiovascular patients, we observed a weak inverse association of cotinine with neopterin, KTR and several kynurenines [41] – in accordance with results published by others [9,14] – and possibly attributable to decreased IFN-γ activity in smokers [42]. The effect of smoking on the immune response and thereby the kynurenine pathway is multi-faceted, and may reflect the opposing nature of cigarette smoking as a proinflammatory factor and the immunosuppression mediated by nicotine [25].

Strengths and limitations

This is the largest community-based study investigating biological and lifestyle determinants of plasma levels of neopterin, KTR and kynurenines. The large sample size and comprehensive data on a large panel of kynurenines and lifestyle factors are unique. The observed plasma concentrations were similar to those reported in another large cohort study [41]. In addition to self-reported smoking behaviour, plasma cotinine provided reliable information on recent nicotine exposure. The cohort enabled us to compare levels of kynurenines and related markers of inflammation between two distinct age groups (46–47 and 70–72 years). However, we could not evaluate the effect of age as a continuous variable, or in other age groups. Lastly, the associations with physical activity might be attenuated, as physical activity was not assessed using a validated physical activity questionnaire. Nevertheless, to the extent of our knowledge, this is the first study that addresses habitual physical activity as a determinant of plasma neopterin, KTR and kynurenines.

Conclusions

Neopterin and KTR are both markers of cellular immune activation, whereas some kynurenines have immune modulatory effects. We observed strong positive associations between these markers and metabolites with age and renal function, indicating that neopterin, KTR and the kynurenines are sufficiently responsive indices to capture the low-grade inflammation that occurs in the elderly. Additionally, KTR and most kynurenines were higher in overweight/obesity, and several kynurenines were associated inversely with smoking. The data also demonstrate that KTR and most kynurenines may reflect the low-grade inflammation present in obese subjects, whereas the inverse association between several kynurenines and smoking potentially reflects the complex effect of smoking in immune functions. Such knowledge highlights potential confounding in epidemiological and clinical studies, but also motivates the inclusion of markers of cellular immunity to disentangle various components of systemic inflammation in the pathogenesis of chronic diseases such as cardiovascular disease and cancer.

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Disclosure

The authors declare that there are no conflicts of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Distribution of neopterin, kynurenine/tryptophan ratio (KTR), tryptophan (Trp) and kynurenines in the total population and stratified by age and gender, the Hordaland Health Study.