# Associations of Plasma Kynurenines With Risk of Acute Myocardial Infarction in Patients With Stable Angina Pectoris

Eva Ringdal Pedersen, Nora Tuseth, Simone J.P.M. Eussen, Per Magne Ueland, Elin Strand, Gard Frodahl Tveitevåg Svingen, Øivind Midttun, Klaus Meyer, Gunnar Mellgren, Arve Ulvik, Jan Erik Nordrehaug, Dennis W. Nilsen, Ottar Nygård

***Objective***—Enhanced tryptophan degradation, induced by the proinflammatory cytokine interferon-γ, has been related to cardiovascular disease progression and insulin resistance. We assessed downstream tryptophan metabolites of the kynurenine pathway as predictors of acute myocardial infarction in patients with suspected stable angina pectoris. Furthermore, we evaluated potential effect modifications according to diagnoses of pre-diabetes mellitus or diabetes mellitus.

***Approach and Results***—Blood samples were obtained from 4122 patients (median age, 62 years; 72% men) who underwent elective coronary angiography. During median follow-up of 56 months, 8.3% had acute myocardial infarction. Comparing the highest quartile to the lowest, for the total cohort, multivariable adjusted hazard ratios (95% confidence intervals) were 1.68 (1.21–2.34), 1.81 (1.33–2.48), 1.68 (1.21–2.32), and 1.48 (1.10–1.99) for kynurenic acid, hydroxykynurenine, anthranilic acid, and hydroxyanthranilic acid, respectively. The kynurenines correlated with phenotypes of the metabolic syndrome, and risk associations were generally stronger in subgroups classified with pre-diabetes mellitus or diabetes mellitus at inclusion ($P_{int}$≤0.05). Evaluated in the total population, hydroxykynurenine and anthranilic acid provided statistically significant net reclassification improvements (0.21 [0.08–0.35] and 0.21 [0.07–0.35], respectively).

***Conclusions***—In patients with suspected stable angina pectoris, elevated levels of plasma kynurenines predicted increased risk of acute myocardial infarction, and risk estimates were generally stronger in subgroups with evidence of impaired glucose homeostasis. Future studies should aim to clarify roles of the kynurenine pathway in atherosclerosis and glucose metabolism. **(*Arterioscler Thromb Vasc Biol*. 2015;35:455-462. DOI: 10.1161/ATVBAHA.114.304674.)**

**Key Words:** acute myocardial infarction ■ atherosclerosis ■ diabetes mellitus ■ epidemiology ■ inflammation ■ insulin resistance ■ tryptophan

Diabetes mellitus (DM) type 2 is a major risk factor for coronary artery disease (CAD) and both DM and CAD are characterized by chronic low-grade inflammation.[1,2] T lymphocytes and macrophages contribute actively to the growth and disruption of atherosclerotic plaques and hence to clinical manifestations such as acute myocardial infarction (AMI).[2] Moreover, inflammatory cells within adipose tissue mediate insulin resistance,[3] an early feature in the progression from normoglycemia to overt type 2 DM.[4]

The proinflammatory cytokine, interferon-γ (IFN-γ), is released from activated $CD4^+$ T cells.[5] IFN-γ induces indoleamine 2,3-dioxygenase, which is the rate-limiting enzyme of the kynurenine (Kyn) pathway.[6] Through this route, the essential amino acid tryptophan (Trp) is metabolized into Kyn and several downstream metabolites, collectively referred to as kynurenines. Hence, the Kyn:Trp ratio (KTR) is an established marker of IFN-γ–mediated (Th1) immune responses.[7]

Previously, we have shown that elevated plasma levels of KTR and an alternative IFN-γ marker, neopterin, were associated with unfavorable prognosis in patients with stable angina pectoris[8] as well as in healthy, elderly adults.[9] Moreover, we identified urine KTR as a particularly strong predictor of coronary events and mortality after elective coronary angiography.[10]

The degradation of Kyn occurs through multiple steps, several of which use vitamin $B_6$ as a cofactor (Figure 1).

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From the Department of Clinical Science (E.R.P., N.T., P.M.U., E.S., G.F.T.S., G.M., J.E.N., D.W.N., O.N.) and Department of Global Public Health and Primary Health Care (S.J.P.M.E.), University of Bergen, Bergen, Norway; Department of Heart Disease, Haukeland University Hospital, Bergen, Norway (N.T., O.N.); Department of Epidemiology, School for Public Health and Primary Care-CAPHRI, Maastricht University, Maastricht, The Netherlands (S.J.P.M.E.); Laboratory of Clinical Biochemistry (P.M.U.) and Hormone Laboratory (G.M.), Haukeland University Hospital, Bergen, Norway; Bevital AS, Bergen, Norway (P.M.U., Ø.M., K.M., A.U.); Department of Cardiology, Stavanger University Hospital, Stavanger, Norway (J.E.N., D.W.N.); and KG Jebsen Center for Diabetes Research, Bergen, Norway (G.M., O.N.).

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Correspondence to Eva Ringdal Pedersen, MD, PhD, Department of Clinical Science, University of Bergen, 5021 Bergen, Norway. E-mail eva.pedersen@k2.uib.no

| Nonstandard Abbreviations and Acronyms | |
|---|---|
| **AA** | anthranilic acid |
| **AMI** | acute myocardial infarction |
| **CAD** | coronary artery disease |
| **CRP** | C-reactive protein |
| **DM** | diabetes mellitus |
| **HAA** | hydroxyanthranilic acid |
| **HK** | hydroxykynurenine |
| **IFN-γ** | interferon-γ |
| **KA** | kynurenic acid |
| **KTR** | kynurenine:tryptophan ratio |
| **Kyn** | kynurenine |
| **Trp** | tryptophan |

Experimental studies suggest that dysregulation of the Kyn pathway may be involved in the pathogenesis of both CAD[11] and DM.[12] However, Trp catabolites other than Kyn have been related only to a limited extent to clinical outcomes in humans, and there is a paucity of data from large-scale epidemiological studies. Thus, we evaluated plasma levels of kynurenic acid (KA) hydroxykynurenine (HK), anthranilic acid (AA), xanthurenic acid, and hydroxyanthranilic acid (HAA) as predictors of AMI in a prospective cohort of patients with suspected stable angina pectoris. In particular, we were interested in whether any associations with adverse prognosis were modified by evidence of impaired glucose homeostasis.

## Materials and Methods

Materials and methods are available in the online-only Data Supplement.

## Results

For the 4122 patients in the current study, median (25th–75th percentile) age at inclusion was 62 (55–70) years and 2967 (72.0%) were men. According to the most recent diagnostic criteria, 1603 (38.9%) had DM, of which the vast majority (97.4%) was classified with type 2 (n=1566). However, only a subset was prescribed antidiabetic medications (Table 1). All together, 1078 (25.9%) of patients were current smokers, 1935 (46.9%) had hypertension, and 1644 (40.4%) reported a prior AMI. Compared with the total population, median age and body mass index were higher in patients with DM, as were the prevalence of hypertension at inclusion and the incidence of AMI during follow-up (Table 1).

### Kynurenines and Baseline Characteristics

Median values of all kynurenines were lower in women than in men ($P<0.001$). KA, HK, and AA were associated positively with age ($\rho \geq 0.19$; $P<0.001$), and except for HAA, kynurenines were higher in patients with DM as compared with subjects with normal glucose metabolism (Table 1). After adjustment for age and sex, median kynurenine-levels were uniformly lower in smokers than in nonsmokers and higher in hypertensive than in normotensive patients (all $P<0.001$). Plasma levels of kynurenines correlated positively with body mass index, serum creatinine, serum triglycerides, and plasma neopterin (Figure 2A). HK was positively correlated with C-reactive protein (CRP; $\rho=0.25$; $P<0.001$), whereas other kynurenines showed only weak or nonsignificant associations with this inflammation marker (Figure 2A). Plasma HAA correlated positively to nonfasting glucose ($\rho=0.21$; $P<0.001$). In contrast, neither metabolite was associated with glycosylated haemoglobin (HbA1c) levels ($\rho \leq 0.04$; $P \geq 0.06$; Figure 2A).

However, in a subgroup of 593 fasting, nondiabetic patients, kynurenines were related to impaired insulin sensitivity, as evaluated by homeostatic model assessments of β-cell function and insulin resistance (Figure 2B). The strongest correlations were found for HAA ($\rho=0.22$ and $\rho=0.34$, respectively; $P<0.001$). Notably, such associations could not be demonstrated for the inflammation markers, neopterin or CRP ($\rho \leq 0.07$, $P \geq 0.09$), and were only weak for the KTR ($\rho \leq 0.08$; $P \geq 0.04$).

### Kynurenines and Risk of AMI in the Total Population

During a median (25th–75th percentile) follow-up time of 56 (44–70) months, 8.3% (n=343) had AMI. Table I in the online-only Data Supplement shows the age- and sex-adjusted associations of kynurenines with risk of AMI. Multivariable hazard ratios (95% confidence interval) comparing the fourth to the first quartile of plasma concentrations were 1.68 (1.21–2.34) for KA, 1.81 (1.33–2.48) for HK, 1.68 (1.21–2.32) for AA, and 1.48 (1.10–1.99) for HAA (Table 2, left). These estimates were comparable with those of the established inflammation markers KTR (1.76 [1.27–2.45]) and CRP (1.45 [1.05–2.00]).

Adding creatinine or CRP to the multivariable model only minimally affected hazard ratios for the individual kynurenines (Tables II and III in the online-only Data Supplement, respectively). Adjustment for KTR somewhat attenuated the risk estimates, which, however, remained statistically significant (Table IV in the online-only Data Supplement). Plasma levels of xanthurenic acid showed no significant associations with AMI risk overall or in subgroups according to DM status (Table I in the online-only Data Supplement; Table 2).

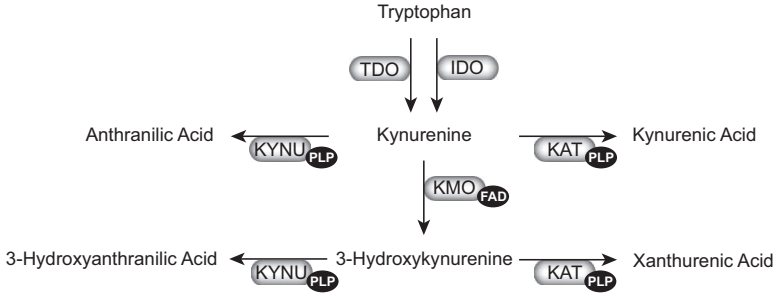


**Figure 1.** Overview of the kynurenine pathway of tryptophan metabolism. FAD indicates flavin adenine dinucelotide (vitamin $B_2$); IDO, indoleamine-2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine-3-monooxygenase; KYNU, kynureninase; PLP, pyridoxal 5′-phosphate (vitamin $B_6$); and TDO, tryptophan 2,3-dioxygenase.

**Table 1. Baseline Characteristics for the Total Population (n=4122) According to Diabetes Mellitus Status**

| | Total (n=4122) | No Diabetes Mellitus (n=1408) | Pre-Diabetes Mellitus (n=1111) | Diabetes Mellitus (n=1603) | $P_{trend}$ |
|---|---|---|---|---|---|
| Male sex, n (%) | 2967 (72.0) | 1055 (74.9) | 787 (70.8) | 1125 (70.2) | 0.005 |
| Age, y | 62 (55–70) | 61 (54–69) | 62 (55–70) | 62 (55–70) | 0.06 |
| BMI, kg/m$^2$ | 26.3 (24.2–28.9) | 25.9 (23.9–28.2) | 26.2 (24.0–28.6) | 26.9 (24.5–29.7) | <0.001 |
| LVEF, % | 65 (60–70) | 66 (60–70) | 66 (60–70) | 65 (60–70) | 0.01 |
| Hypertension, n (%) | 1935 (46.9) | 600 (42.6) | 513 (46.2) | 822 (51.3) | <0.001 |
| Current smoking, n (%) | 1070 (26.0) | 375 (26.6) | 278 (25.0) | 417 (26.0) | 0.72 |
| Plasma biomarkers related to the kynurenine pathway | | | | | |
| Tryptophan, μmol/L | 70.2 (60.7–79.7) | 71.0 (62.2–80.1) | 69.4 (60.3–78.6) | 70.2 (59.7–80.1) | 0.35 |
| Kynurenine, μmol/L | 1.68 (1.39–2.01) | 1.68(1.39–1.98) | 1.65 (1.37–1.98) | 1.71 (1.40–2.06) | 0.14 |
| Kynurenic acid, nmol/L | 48.0 (37.1–62.5) | 47.9 (37.1–61.8) | 46.5 (36.0–60.7) | 49.4 (37.7–64.5) | 0.03 |
| Anthranilic acid, nmol/L | 14.4 (11.4–18.4) | 14.1 (11.1–18.0) | 14.2 (11.3–17.5) | 15.0 (11.8–19.2) | <0.001 |
| Hydroxykynurenine, nmol/L | 30.8 (24.0–39.9) | 29.2 (23.1–37.5) | 31.3 (24.7–39.5) | 32.3 (24.5–42.5) | <0.001 |
| Xanthurenic acid, nmol/L | 14.4 (10.2–20.1) | 14.0 (10.1–19.8) | 14.2 (9.8–19.9) | 14.8 (10.5–20.5) | 0.005 |
| Hydroxyanthranilic acid, nmol/L | 34.2 (25.9–45.2) | 34.6 (26.4–45.2) | 33.6 (25.5–43.8) | 34.2 (25.5–46.3) | 0.60 |
| Pyridoxal phosphate, nmol/L | 41.3 (29.5–59.8) | 43.9 (30.8–61.4) | 39.7 (29.0–57.7) | 40.3 (28.9–58.5) | <0.001 |
| Inflammation markers | | | | | |
| Serum C-reactive protein, mg/L | 1.8 (0.9–1.0) | 1.7 (0.8–3.2) | 1.7 (0.9–3.7) | 1.9 (0.9–4.1) | <0.001 |
| Plasma neopterin, nmol/L | 8.2 (6.7–10.4) | 8.1 (6.6–10.1) | 8.1 (6.7–10.2) | 8.3 (6.7–10.8) | 0.09 |
| Plasma KTR, nmol/μmol | 23.8 (19.8–29.0) | 23.3 (19.8–28.2) | 23.9 (19.6–29.1) | 24.4 (20.0–29.7) | <0.001 |
| Serum lipids | | | | | |
| ApoA1, g/L | 1.30 (1.13–1.48) | 1.31 (1.14–1.49) | 1.30 (1.14–1.48) | 1.28 (1.12–1.47) | 0.009 |
| ApoB, g/L | 0.87 (0.73–1.04) | 0.88 (0.74–1.05) | 0.87 (0.72–1.04) | 0.85 (0.73–1.03) | 0.06 |
| Triglycerides, nmol/L | 1.50 (1.08–2.14) | 1.42 (1.05–2.05) | 1.46 (1.07–2.00) | 1.59 (1.12–2.36) | <0.001 |
| Parameters of kidney function | | | | | |
| Serum creatinine | 89 (81–98) | 89 (81–98) | 89 (81–98) | 89 (80–99) | 1.0 |
| eGFR, mL/min per 1.73 m2 | 91 (78–99) | 91 (80–100) | 91 (79–99) | 90 (76–99) | 0.13 |
| Parameters of glucose homeostasis | | | | | |
| Nonfasting glucose, mmol/L | 5.6 (5.1–6.6) | 5.4 (4.9–6.0) | 5.5 (5.0–6.1) | 6.1 (5.3–8.4) | 0.01 |
| HbA1c, % | 6.1 (5.4–6.9) | 5.1 (4.6–5.4) | 6.1 (5.9–6.3) | 7.1 (6.7–7.8) | 0.003 |
| Serum C-peptide, pmol/L* | 706 (526–976) | 706 (526–989) | 719 (523–963) | 822 (604–1156) | <0.001 |
| Serum insulin, pmol/L* | 20.8 (19.7–55.0) | 26.5 (19.7–60.2) | 19.7 (19.7–52.9) | 35.3 (19.7–87.2) | <0.001 |
| HOMA2, insulin† | | | | | |
| β-Cell activity, % | 53.9 (44.4–79.2) | 56.8 (45.0–87.1) | 51.7 (43.3–70.9) | NA | 0.06 |
| Insulin sensitivity, % | 250 (92.4–266) | 200 (86.1–265) | 256 (97.7–266) | NA | 0.20 |
| Insulin resistance | 0.40 (0.40–1.10) | 0.50 (0.40–1.20) | 0.40 (0.40–1.00) | NA | 0.14 |
| Cardiovascular history | | | | | |
| Prior AMI, n (%) | 1664 (40.4) | 572 (40.6) | 420 (37.8) | 672 (41.9) | 0.43 |
| Angiographic extent of CAD | | | | | |
| No significant CAD,‡ n (%) | 1040 (25.2) | 316 (22.4) | 316 (28.4) | 408 (25.5) | 0.07 |
| One-vessel disease, n (%) | 952 (23.1) | 359 (25.5) | 238 (21.4) | 355 (22.2) | 0.03 |
| Two-vessel disease, n (%) | 918 (22.3) | 311 (22.1) | 265 (23.9) | 342 (21.3) | 0.59 |
| Three-vessel disease, n (%) | 1212 (29.4) | 422 (30.0) | 292 (26.3) | 498 (31.1) | 0.46 |
| Revascularization after baseline coronary angiography | | | | | |
| PCI, n (%) | 1335 (32.4) | 471 (33.5) | 361 (32.5) | 503 (31.4) | 0.23 |
| CABG, n (%) | 890 (21.6) | 318 (22.6) | 228 (20.5) | 344 (21.5) | 0.48 |
| Medications at discharge | | | | | |
| β-Blockers, n (%) | 2986 (72.4) | 1048 (74.4) | 779 (70.1) | 1159 (72.3) | 0.22 |
| ACEI or an ARB, n (%) | 1320 (32.0) | 369 (26.2) | 354 (31.9) | 597 (37.2) | <0.001 |

(*Continued*)

**Table 1. Continued**

| | Total (n=4122) | No Diabetes Mellitus (n=1408) | Pre-Diabetes Mellitus (n=1111) | Diabetes Mellitus (n=1603) | $P_{trend}$ |
|---|---|---|---|---|---|
| Statins, n (%) | 3303 (80.1) | 1150 (81.7) | 869 (78.2) | 1284 (80.1) | 0.31 |
| Aspirin, n (%) | 3366 (81.7) | 1199 (85.2) | 884 (79.6) | 1283 (80.0) | <0.001 |
| Insulin, n (%) | 142 (3.4) | 0 (0) | 0 (0) | 142 (8.9) | … |
| Sulfonylureas, n (%) | 169 (4.1) | 0 (0) | 0 (0) | 169 (10.5) | … |
| Metformin, n (%) | 193 (4.7) | 0 (0) | 0 (0) | 193 (12.0) | … |
| Other antidabetic drugs, n (%) | 15 (0.4) | 0 (0) | 0 (0) | 15 (0.9) | … |
| Clinical end points during follow-up | | | | | |
| AMI, n (%) | 343 (8.3) | 99 (7.0) | 79 (7.1) | 165 (10.3) | 0.001 |

ACEI indicates angiotensin-converting enzyme inhibitors; AMI, acute myocardial infarction; ARB, angiotensin II receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; Hba1c, glycosylated haemoglobin; HOMA, homeostatic model assessment; KTR, kynurenine:tryptophan ratio; LVEF, left ventricular ejection fraction; and PCI, percutaneous coronary intervention.

*Measured in fasting patients only (n=1004).

†Calculated in fasting, nondiabetic patients only (n=593).

‡Angiographically normal coronary artery or plaque(s) with <50% luminal narrowing.

### Sensitivity Analyses

Because of the different routines for sample handling at the 2 study centers, separate Cox analyses were performed for patients included at Haukeland University Hospital (n=3384) and Stavanger University Hospital (n=738). Risk estimates were similar to those found in the whole data set (data not shown). Similarly, including adjustment for study center to the multivariable model did not affect the risk estimates for the total population (Table V in the online-only Data Supplement). There was no statistically significant interaction between study center and any of the kynurenines ($P_{int} \geq 0.17$) in relation to AMI risk.

### Kynurenines and Risk of AMI According to DM Status

We further investigated potential effect modifications according to DM status (Table 2, right; Figure I in the online-only Data Supplement). The associations of kynurenines with incident AMI were generally stronger in those classified with pre-DM or DM than in the subgroup without evidence of impaired glucose metabolism. Comparing quartile 4 versus quartile, hazard ratio s (95% confidence interval) among patients with pre-DM were 2.51 (1.27–4.95), 2.24 (1.18–4.24), and 2.27 (1.20–4.33) for KA, AA, and HAA, respectively. In diabetics, the strongest risk estimate was found for HK (2.37 ([1.43–3.93]).

### Goodness of Fit, Risk Reclassification, and Discrimination

Evaluated in the total population, the addition of KA, HK, AA, or HAA improved goodness of fit for the multivariable model (Table 3). HK and AA both provided significant net reclassification improvements (95% confidence interval) of 0.21 (0.08–0.35) and 0.21 (0.07–0.35), respectively. However, the increments in areas under receiver operator characteristic curves were modest and statistically significant for AA only (Table 3). These results were literally unchanged after including CRP to the multivariable model (Table VI in the online-only Data Supplement).

Among patients without DM, HK was the only metabolite improving model goodness of fit ($P$=0.03). With the exception of xanthurenic acid, all kynurenines provided significant reductions of Aikaikes' Information Criteria values in patients with pre-DM ($P \leq 0.05$), whereas HK, AA, and HAA improved model fit in patients with DM ($P \leq 0.01$).

Reclassification analyses showed similar results for AA and HK in stratified analyses as in the total population, with only weak tendencies toward numerically stronger estimates among patients with pre-DM or DM (net reclassification improvements, 0.20–0.28). In addition, HAA, which did not improve risk categorization in the total population, provided a net reclassification improvement (95% confidence interval) of 0.26 (0.06–0.49) among patients with pre-DM.

Among nondiabetic patients, the area under receiver operator characteristic curve for the multivariable model without individual kynurenines was 0.66. Each of the metabolites provided increases in areas in the range of 0.002 to 0.009. Corresponding estimates were 0.73 (increments by kynurenines, 0.009–0.026) and 0.74 (increments by kynurenines, 0.003–0.010) for patients with pre-DM and DM, respectively. In subgroup analyses, none of the increases in areas under the curves were statistically significant ($P \geq 0.08$), probably because of reduced power.

## Discussion

### Principal Findings

In patients with suspected stable CAD, downstream Trp metabolites of the Kyn pathway were associated with increased risk of incident AMI. Plasma kynurenines were generally higher in patients with DM than in subjects with normal glucose metabolism. Levels were associated with several components of the metabolic syndrome (hypertension, body mass index, and serum triglycerides) but showed only weak or no correlations to CRP, glucose, or HbA1c values. Notably, the associations with adverse outcome remained significant after adjustment for potential confounders. Moreover, risk estimates were significantly higher in patients with evidence of impaired glucose homeostasis at baseline.

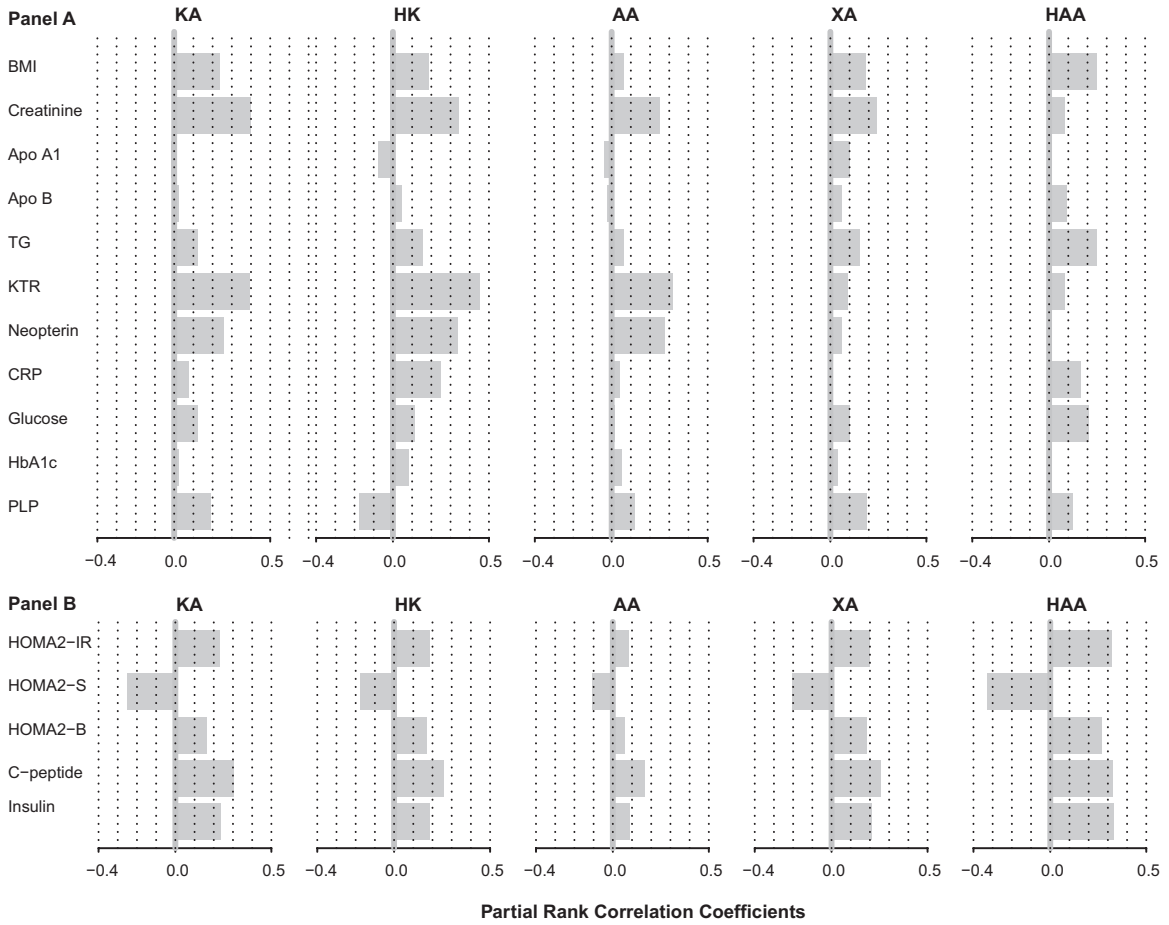


**Figure 2.** Associations of plasma kynurenines with selected baseline variables. Age-and sex-adjusted Spearman rank correlations are reported for the total population (n=4122; **A**) and for a subgroup of fasting, nondiabetic patients (n=593; **B**). Statistical significance at the $P \leq 0.001$ level is achieved at $\rho \geq 0.06$, and $\rho \geq 0.14$, for the parameters of **A** and **B**, respectively. AA indicates anthranilic acid; BMI, body mass index; CRP, C-reactive protein; HAA, hydroxyanthranilic acid; HbA1c, glycosylated haemoglobin; HK, hydroxykynurenine; HOMA2-B, HOMA2-IR, HOMA2-S, homeostatic model assessments for β-cell activity, insulin resistance, and insulin sensitivity, respectively; KA, kynurenic acid; KTR, kynurenine:tryptophan ratio; PLP, pyridoxal 5′-phosphate (vitamin $B_6$); TG, triglycerides; and XA, xanthurenic acid.

## Kynurenines, Atherosclerosis, and Insulin Resistance

Similar to our findings among patients with stable angina pectoris, associations of kynurenines with CAD risk factors have been reported in presumably healthy populations.[13,14] HK correlated with intima media thickness of the carotids.[15] Moreover, HK and KA were both associated with the presence of cardiovascular disease in patients with renal failure.[16] Interestingly, a recent metabolomics study reported correlations of kynurenines with components of the metabolic syndrome[17] and hypothesized these metabolites to be involved in the pathogenesis of insulin resistance. To the best of our

**Table 2. Associations* of Plasma Kynurenines With Risk of Acute Myocardial Infarction in the Total Study Population and Stratified According to Diabetes Status**

| | Total (n=4122) | | | | | No Diabetes Mellitus (n=1408) | Pre-Diabetes Mellitus (n=1111) | Diabetes Mellitus (n=1603) | |
|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | | | | | HR (95% CI) | | | |
| Biomarker | $Q_1$ | $Q_2$ | $Q_3$ | $Q_4$ | $P_{trend}$ | $Q_4$ vs $Q_1$ | $Q_4$ vs $Q_1$ | $Q_4$ vs $Q_1$ | $P_{int}$ |
| KA | 1.00 | 1.14 (0.81–1.64) | 1.35 (0.97–1.89) | 1.68 (1.21–2.34) | 0.001 | 1.31 (0.72–2.40) | 2.51 (1.27–4.95) | 1.52 (0.92–2.49) | 0.03 |
| HK | 1.00 | 0.83 (0.58–1.19) | 1.22 (0.87–1.69) | 1.81(1.33–2.48) | <0.001 | 1.21 (0.69–2.11) | 1.98 (1.03–3.79) | 2.37 (1.43–3.93) | 0.05 |
| AA | 1.00 | 1.03 (0.72–1.45) | 1.37 (0.98–1.90) | 1.68 (1.21–2.32) | <0.001 | 1.12 (0.93–1.34) | 2.24 (1.18–4.24) | 1.84 (1.09–3.11) | 0.02 |
| XA | 1.00 | 0.96 (0.71–1.30) | 0.88 (0.64–1.20) | 1.14 (0.84–1.55) | 0.49 | 0.85 (0.45–1.58) | 1.29 (0.70–2.39) | 1.23 (0.80–1.90) | 0.27 |
| HAA | 1.00 | 0.88 (0.63–1.22) | 1.04 (0.76–1.42) | 1.48 (1.10–1.99) | 0.005 | 0.97 (0.53–1.76) | 2.27 (1.20–4.33) | 1.49 (0.97–2.27) | 0.01 |

HRs are presented per quartile increment and for quartile 4 ($Q_4$) vs quartile 1 ($Q_1$) of the respective biomarkers. AA indicates anthranilic acid; CI, confidence interval; HAA, hydroxyanthranilic acid; HK, hydroxykynurenine; HR, hazard ratio; KA, kynurenic acid; and XA, xanturenic acid.

*Adjusted for age, sex, body mass index, hypertension, diabetes mellitus, smoking, angiographic extend of coronary artery disease, apoA1, and apoB.

**Table 3. Model Fit, Reclassification, and Discrimination Indices for the Total Study Population (n=4122)**

| | Acute Myocardial Infarction | | | | | |
|---|---|---|---|---|---|---|
| | AIC | *P* Value | NRI (95% CI) | *P* Value | ROC-AUC | *P* Value |
| Model* without biomarker | 5267 | … | … | … | 0.705 | … |
| Model* with KA | 5262 | 0.01 | 0.08 (–0.05 to 0.22) | 0.24 | 0.706 | 0.62 |
| Model* with HK | 5246 | <0.001 | 0.21 (0.08 to 0.35) | 0.002 | 0.715 | 0.06 |
| Model* with AA | 5259 | 0.002 | 0.21 (0.07 to 0.35) | 0.002 | 0.714 | 0.02 |
| Model* with HAA | 5260 | 0.003 | 0.08 (–0.05 to 0.21) | 0.24 | 0.712 | 0.07 |

AA indicates anthranilic acid, AIC, Akaike's Information criteria; CI, confidence interval; HAA, hydroxyanthranilic acid; HK, hydroxykynurenine; KA, kynurenic acid; NRI, net reclassification improvement; and ROC-AUC, area under receiver operator characteristics curve.

*Including age, sex, body mass index, hypertension, diabetes mellitus, smoking, angiographic extend of coronary artery disease, and serum levels of apoA1 and apoB.

knowledge, however, downstream Trp catabolites have not been evaluated prospectively in large-scale epidemiological studies.

## Possible Mechanisms

The expression of genes related to Kyn metabolism was upregulated in atherosclerotic plaques.[11] Further, Trp degradation was increased in fat tissues and liver of obese as compared with lean women,[18] supporting a role of this metabolic pathway in CAD and other obesity-associated pathologies.

In the present study, downstream kynurenines were strongly correlated with KTR, an established marker of IFN-γ activity and Th1 immune responses. Notably, their risk estimates remained statistically significant even after adjustment for plasma KTR levels. Hence, the associations with incident AMI seem not solely to reflect IFN-γ activation per se but may suggest independent pathogenic roles of the downstream metabolites.

Trp catabolism is considered to be centrally involved in balancing activation and inhibition of the immune system.[19] In a mouse model, treatment with HAA reduced atherosclerotic lesions in the aorta, modulated local as well as systemic inflammatory responses, and lowered lipoprotein levels.[20] This metabolite and other kynurenines were also identified as endogenous ligands of the transcription factor aryl hydrocarbon receptor known to mediate vascular inflammation and procoagulant effects.[21]

Our findings of positive correlations of kynurenines to creatinine are in line with previous publications and possibly reflect the combination of increased synthesis because of inflammatory activation[22] and reduced renal clearance[23] in patients with chronic kidney disease. Indeed, Trp metabolism has been hypothesized as a causal mechanism contributing to accelerated atherosclerosis in renal disease.[21,24] Notably, however, adjustment for serum creatinine levels had only minor effects on our risk estimates. Hence, the associations of kynurenines with outcomes seem not solely to reflect pathways activated in renal dysfunction.

Individual kynurenines inhibited proinsulin synthesis from pancreatic islets in rat models[25] and formed complexes with insulin reducing its biological activity.[12] Impaired insulin sensitivity may be present years before the occurrence of overt hyperglycemia[4] and is associated with increased risk of macrovascular disease even in the absence of type 2 DM.[4]

Impaired endothelial function represents a common feature of atherosclerosis, renal dysfunction, and insulin resistance[26] and is characterized by decreased NO-mediated vasodilatation of arteries.[2] Oxidative stress is a key pathogenic factor.[26] In an environment with overload of free radicals, arginine metabolism is shifted from NO synthesis toward the production of superoxide anion in a self-perpetuating process.[27] HK and HAA potentially contribute to this process by promoting lipid peroxidation[12] as well as by inhibiting NO synthase.[28] Conversely, peroxynitrite, formed from NO during oxidative stress, is able to bind and inactivate the indoleamine-2,3 deoxygenase enzyme.[29] Moreover, Kyn has been identified as a potent, NO-independent, vasodilator.[30] Vascular protective effects have also been revealed for KA.[31] Hence, evidence suggests that Trp degradation has diverse and complex effects on inflammatory pathways, metabolism, and the vasculature.

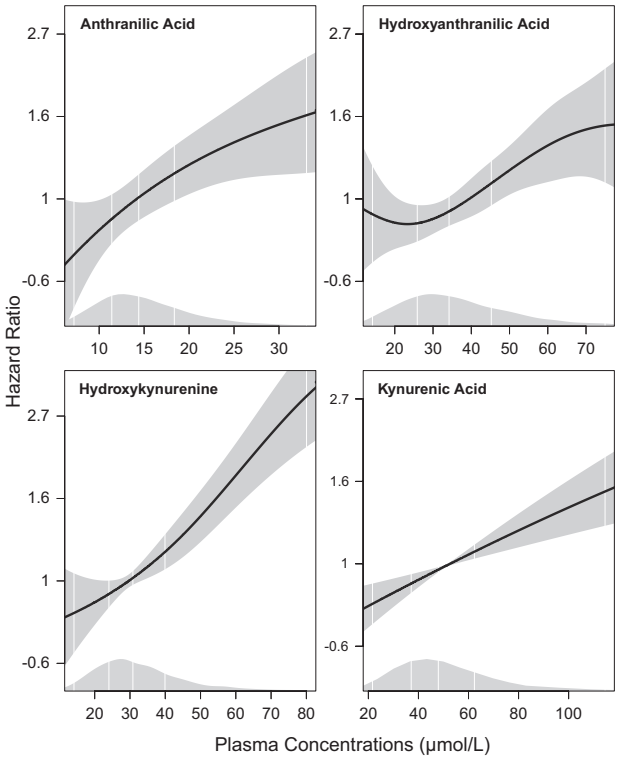


**Figure 3.** Dose–response relationship between plasma kynurenines and risk of acute myocardial infarction for the total population. The regression models are adjusted for age, sex, body mass index, hypertension, smoking, diabetes mellitus, apoA1, apoB, and angiographic extent of coronary artery disease. The solid lines show the hazard ratios and the shaded areas 95% confidence intervals. Density plots show the distributions of plasma kynurenines and vertical lines denote the 10th, 25th, 50th, 75th, and 90th percentiles.

The correlations of kynurenines to the homeostatic model assessment of insulin resistance support previous studies linking Trp degradation to impaired insulin sensitivity.[17] Interestingly, similar associations were also observed for several branched chained amino acids.[32] Our findings thus possibly mirror a more generalized derangement of amino acid metabolism in the insulin-resistant state.[33] Hence, despite extensive experimental data suggesting kynurenines as potential active mediators, it is still unclear whether elevated plasma levels reflect causal pathways or only epiphenomena of disease development.

Several single nucleotide polymorphisms for genes of the Kyn pathway are identified.[34,35] To the best of our knowledge, such genetic variants have not been related to cardiovascular or diabetic outcomes in humans. Future Mendelian randomization studies may extend current knowledge on causality. This may be of particular value because the Kyn axis can eventually be targeted by lifestyle[36] or medical[25] intervention.

### Strengths and Limitations

Strengths of the study include its prospective design and large sample size. We had detailed data on clinical baseline characteristics including parameters of glucose metabolism as well as the angiographic extent of CAD. Follow-up was ascertained through the use of a patient administrative and a population- based registry. We cannot exclude the possibility that the report of clinical end points has been subjected to some under-reporting or other misclassification. However, we do not suspect that any misclassifications differ according to the levels of the kynurenines.

The homeostatic model assessments of insulin resistance and β-cell activity could only be calculated reliably in a subgroup reporting to be fasting at the time of blood sampling. Further limitations of our study include potential bias introduced by the single measurement of biomarkers. Although HbA1c has low intraindividual variability,[37,38] current guidelines recommend glucose or HbA1c to be determined at 2 separate occasions for the diagnosis of DM.[39] Notably, a prior publication from our group demonstrated that all kynurenines have sufficient within-person reproducibility to allow 1-exposure assessment in epidemiological studies.[40]

The significant associations of kynurenines with AMI risk persisted even after extensive multivariable adjustment. However, improvements in risk classifications by these metabolites were only moderate in the total population as well as in subgroups with impaired glucose homeostasis. Hence, it remains to be determined whether their measurements can be justified in clinical practice. Our data nonetheless provide epidemiological support to experimental studies linking Kyn pathway activation to atherosclerotic complications and insulin resistance.

### Conclusions

In patients with suspected stable angina pectoris, downstream metabolites from the Kyn axis predicted increased risk of AMI independently of traditional risk factors. Individual kynurenines correlated with phenotypes of the metabolic syndrome, and the associations with adverse prognosis were generally stronger among patients with evidence of impaired glucose homeostasis. The roles of Trp degradation in CAD progression and energy metabolism should be further elucidated, as it potentially represents a novel interventional target.[25,36]

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## Disclosures

Dr Midttun is a board member of the Foundation to promote research into functional vitamin $B_{12}$ deficiency. The other authors report no conflicts.

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## Significance

Increased tryptophan degradation induced by the proinflammatory cytokine interferon-γ has been related to coronary artery disease and insulin resistance. We evaluated downstream tryptophan metabolites from the kynurenine pathway in a large-scale prospective cohort study. Among ≈4000 patients with stable angina pectoris, followed up for >4 years, plasma levels of several kynurenines predicted incident acute myocardial infarction independently of traditional risk factors. The kynurenines correlated with phenotypes of the metabolic syndrome. Moreover, risk estimates were generally higher in subgroups with evidence of impaired glucose homeostasis at baseline. Our findings support experimental studies linking the kynurenine pathway to atherogenesis and insulin resistance and strongly encourage further research into pathogenic roles of tryptophan catabolism in lifestyle-related diseases.