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# Purine metabolism is dysregulated in patients with major depressive disorder



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## ABSTRACT

**Introduction:** The purine cycle and altered purinergic signaling have been suggested to play a role in major depressive disorder (MDD). Nevertheless, data on this topic are scarce. Based on previous studies, we hypothesized that compared with non-depressed controls, MDD patients have distinct purine metabolite profiles.

**Methods:** The samples comprised 99 MDD patients and 253 non-depressed controls, aged 20–71 years. Background data were collected with questionnaires. Fasting serum samples were analyzed using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC–MS) to determine seven purine cycle metabolites belonging to the purine cycle. We investigated the levels of these metabolites in three settings: (1) MDD patients vs. non-depressed controls and (2) remitted vs. non-remitted MDD patients, and also (3) within-group changes in metabolite levels during the follow-up period.

**Results:** In logistic regression adjusted for age, gender, smoking, alcohol use, physical exercise, glycosylated hemoglobin, and high-density lipoprotein cholesterol, lower levels of inosine (OR 0.89, 95% CI 0.82–0.97) and guanosine (OR 0.32, 95% CI 0.17–0.59), and higher levels of xanthine (OR 2.21, 95% CI 1.30–3.75) were associated with MDD vs. the non-depressed group. Levels of several metabolites changed significantly during the follow-up period in the MDD group, but there were no differences between remitted and non-remitted groups.

**Conclusions:** We observed altered purine metabolism in MDD patients compared with non-depressed controls. Furthermore, our observations suggest that circulating xanthine may accumulate in MDD patients.

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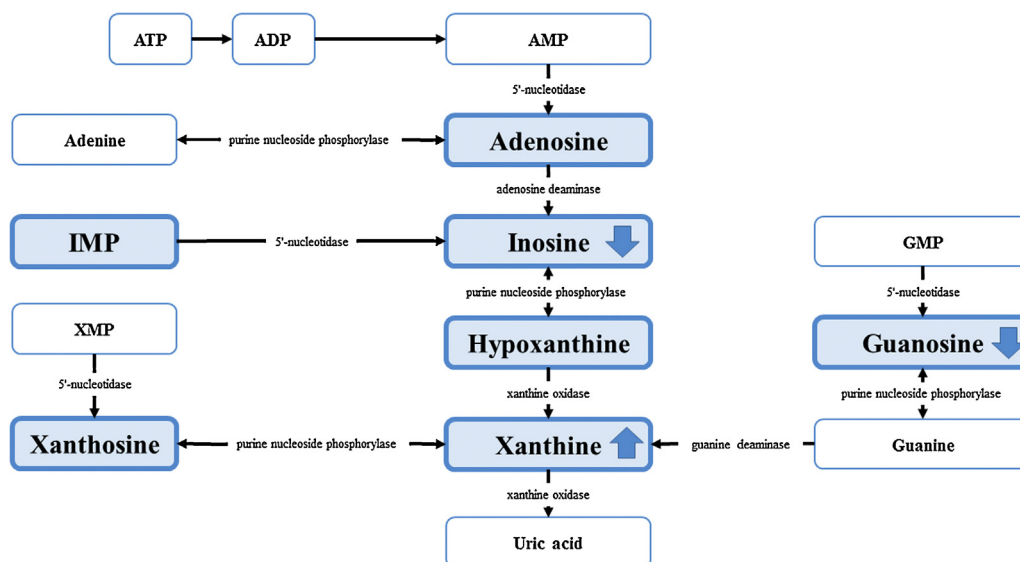
## 1. Introduction

Dysregulation of the purine cycle may be of relevance in major depressive disorder (MDD), a condition that is a major public health concern. In addition, altered metabolic activity of the purine cycle has been linked with several MDD-related systemic responses such as increased pro-inflammatory and oxidative processes (Kaddurah-Daouk et al., 2013, 2011). In the purine cycle,

the purine nucleotides adenosine monophosphate (AMP), inosine 5-monophosphate (IMP), xanthine monophosphate (XMP), and guanosine monophosphate (GMP) are converted to uric acid, a potent antioxidant (Davies et al., 1986) (Fig. 1). Lowered plasma and serum levels of uric acid have been observed in MDD (Chaudhari et al., 2010; Kesebir et al., 2014; Wen et al., 2012). Moreover, lower cerebro-spinal fluid (CSF) levels of hypoxanthine and xanthine, the two metabolites preceding uric acid, have previously been linked with depression (Agren et al., 1983). Additionally, some studies have indicated a correlation between CSF levels of purine metabolites and monoamine metabolites such as homovanillic acid and 5-hydroxyindoleacetic acid, suggesting the parallel metabolism of purines and monoamines in the brain (Kaddurah-Daouk et al.,

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**Fig. 1.** Analyzed metabolites are marked with a darker shade. Arrows represent the levels of the metabolites in the MDD group compared to the non-depressed control group.

2012; Niklasson et al., 1983). Nevertheless, some other studies have failed to support the possible role of the purine cycle in MDD. (Steffens and Jiang, 2010) found no differences in the plasma levels of purine metabolites between depressed and non-depressed patients with heart failure. Similarly, no differences in CSF levels of purine metabolites were detected between individuals with current MDD, remitted MDD, and no MDD (Kaddurah-Daouk et al., 2012).

One of the purine cycle nucleosides, adenosine (the dephosphorylation product of AMP), has widespread neuromodulatory effects in the central nervous system (CNS). In conditions of increased energy need, activation of adenosine A1 receptors leads to inhibition of the neural release of glutamate, dopamine, serotonin, and acetylcholine (Abbracchio et al., 1995; Boison, 2008, 2007; Cunha, 2005). In addition to the CNS, adenosine receptors are systemically expressed, and their activation in the immune system suppresses the secretion of pro-inflammatory cytokines (Haskó et al., 2008), which have been implicated in the pathophysiology of depression (Woo et al., 2015).

Other nucleosides of the purine cycle, inosine and guanosine, also have systemic effects. For example, inosine and guanosine exert an anti-depressive effect by modulating NMDA receptors (Kaster et al., 2013, 2012). Moreover, both of these metabolites have systemic anti-inflammatory effects (Haapakoski et al., 2011; Jiang et al., 2007; Liaudet et al., 2001). Hypoxanthine, xanthine, and uric acid are the three final metabolites of the purine cycle. The conversion of hypoxanthine to xanthine, and xanthine to uric acid, is catabolized by the enzyme xanthine oxidase (XO). Interestingly, MDD patients have displayed elevated levels of XO and adenosine deaminase (ADA), another important enzyme of the purine cycle (Herken et al., 2007). To our best knowledge, no direct information on the blood–brain barrier (BBB) permeability of these purine metabolites is available. However, it is probable that serum levels of measured purine metabolites are correlated with CSF levels, as inosine and uric acid have been demonstrated to permeate the BBB (Bowman et al., 2010; Levine and Morley, 1982). All intermediates of the purine cycle are closely related to either uric acid or inosine in their molecular structure. Moreover, caffeine, which closely resembles adenosine and binds to the same receptors, also permeates the BBB (McCall et al., 1982).

In order to further clarify the role of purine metabolism in MDD, the aim of the present study was to compare the serum

levels of 7 metabolites relevant to purine metabolism (i.e., inosine, xanthine, guanosine, hypoxanthine, xanthosine, adenosine, and IMP) (1) between an MDD group and a non-depressed group, (2) with regards to the remission status of the MDD group, and (3) longitudinally within the remitted and non-remitted groups. We hypothesized that we would observe findings suggesting (1) increased activity of the purine cycle (i.e. decreased levels of adenosine, hypoxanthine, and xanthine; increased levels of inosine) during depression and (2) normalized activity of the purine cycle in the remitted (i.e. similar levels of the measured purine metabolites to the non-depressed control group), but not in the non-remitted group.

## 2. Methods

### 2.1. Study samples

The present study utilized two sample sets: (1) a naturalistic follow-up study sample of patients with MDD (i.e., the NeuroDep Study) and (2) a general population-based sample of non-depressed individuals (i.e., the Lapinlahti Study). The age distribution of all the participants was 20–71 years. Both studies were approved by the Ethics Committee of Kuopio University Hospital. All participants gave written informed consent before entering the study. Both of the used study samples represent the same population, and Kuopio University Hospital serves the municipality area of Lapinlahti.

#### 2.1.1. Patient sample set (the NeuroDep Study)

The study sample set consisted of 99 outpatients with MDD recruited from the Department of Psychiatry at Kuopio University Hospital. At baseline, the diagnosis of MDD was confirmed by using the structured clinical interview for DSM-IV (SCID) (DSM-IV; American Psychiatric Association, 1994). Of the initial 99 patients, 78 participated in the follow-up study (mean follow-up time 8 months; range 5–13 months). We observed no differences in age ( $p=0.152$ ), sex ( $p=0.663$ ), marital status ( $p=0.575$ ), alcohol use ( $p=0.324$ ), smoking ( $p=0.964$ ), regular exercise ( $p=0.964$ ), or BDI scores ( $p=0.493$ ) between the individuals who continued to participate in the study and those who did not.

All participants gave venous blood samples at baseline and on follow-up. The initial exclusion criteria consisted of epilepsy,

bipolar disorder, psychotic disorders, mental symptomology due to substance abuse, and current somatic conditions preventing participation in the study.

At baseline, 84 (84.8%) of the patients used anti-depressive medication and 48 (48.5%) used antipsychotic medication. Antidepressant use was distributed as follows: (1) selective serotonin reuptake inhibitors (SSRI),  $n=42$  (42.4%); (2) venlafaxine,  $n=21$  (21.2%); (3) mirtazapine,  $n=13$  (13.1%); (4) duloxetine,  $n=12$  (12.1%); (5) moclobemide,  $n=8$  (8.9%); (6) bupropion,  $n=6$  (6.1%); and (7) trazodone,  $n=1$  (1.0%). Antipsychotic use was distributed as follows: (1) levomepromazine,  $n=1$  (1.01%); (2) perphenazine,  $n=1$  (1.01%); (3) sertindole,  $n=1$  (1.01%); (4) quetiapine,  $n=28$  (28.23%); (5) clozapine,  $n=1$  (1.01%); (6) olanzapine,  $n=3$  (3.03%); (7) aripiprazole,  $n=1$  (1.01%); and (8) risperidone,  $n=1$  (1.01%).

### 2.1.2. Non-depressed control sample set (the Lapinlahti Study)

The Lapinlahti Study is a population-based follow-up study of 480 individuals living in the municipality area of Lapinlahti, Finland. The sample used in this study was collected as part of the 5-year follow-up of the study in 2010. Altogether, 253 non-depressed controls were derived from the Lapinlahti Study. The exclusion criteria were Beck Depression Inventory (BDI) scores  $\geq 10$ , i.e. an elevated level of depressive symptoms at the time of the study or 5 years earlier (Beck et al., 1961) and reported use of antidepressants. Participants underwent a complete health examination including anthropometric measurements, and completed a background questionnaire (Savolainen et al., 2014).

## 2.2. Background data

The following variables were extracted from the questionnaires completed by the participants in both of the sample sets: the frequency of weekly physical exercise ( $\geq 1$  times vs.  $<1$  time), regular smoking (yes vs. no), weekly alcohol use (0–5 portions vs.  $\geq 6$  portions; 1 portion corresponds to 1 bottle of beer, 1 glass of wine, or 4 cl of spirits), marital status (married or living with a partner vs. living alone), and educational level (university, polytechnic or college education vs. lower than university, polytechnic or college education). Depressive symptoms were assessed with the BDI-21 (Beck et al., 1961). The use of prescription and over-the-counter medications was also recorded with a questionnaire, and double-checked from the prescription documents the patients provided at the study visit.

## 2.3. Laboratory analyses

Before venipuncture, the participants in both study populations were instructed to fast for 12 h. All samples were stored at  $-70^\circ\text{C}$  until analyzed in one batch. The blood samples were used to quantify the concentrations of a batch of metabolites related to different aspects of the NeuroDep and Lapinlahti studies. Based on previous literature indicating a role of purine metabolism in MDD, this study focused on the 7 metabolites of the purine cycle (i.e., inosine, xanthine, guanosine, hypoxanthine, xanthosine, adenosine, and IMP).

Metabolites ( $\mu\text{moles/L}$ ) were extracted from the serum samples using acetonitrile (1:4, sample:solvent) and analyzed using an ACQUITY UPLC-MS/MS system (Waters Corporation, Milford, MA, USA). A detailed protocol and instrument conditions have been published elsewhere (Roman-Garcia et al., 2014).

Serum high density lipoprotein cholesterol (HDL-C; reference value  $1.0\text{mmol/l}$ ) and Hb1Ac measurements were carried out according to the routine protocol in the accredited medical laboratory of Kuopio University Hospital. The analytical protocols have

been described in detail elsewhere (Chang et al., 1998; Siekmann et al., 1976)

## 2.4. Statistical methods

To analyze the differences between groups, we used the chi-squared test and Fisher's exact test for categorical variables, and the Mann-Whitney  $U$  test for continuous variables due to a non-normal distribution. The changes in metabolite levels within the groups during the follow-up period were analyzed using the Wilcoxon signed-rank test. The normality of the distribution for the continuous variables was examined using the Kolmogorov-Smirnov test. Correlations among the measured metabolites were analyzed with Spearman's rank correlation coefficients. To assess whether the dropout of 21 participants could have biased our sample, we analyzed group differences in the baseline between those who participated in follow-up assessments and those who did not.

The analyses were conducted in three separate settings. In setting 1, we compared the purine metabolite levels of the MDD group and non-depressed control group. In setting 2, the comparisons were conducted between remitted vs. non-remitted MDD groups after the follow-up. In setting 3, we compared the within-group changes in metabolite levels from baseline to follow-up.

Logistic regression (method: enter) was used for multivariate analysis. In baseline analyses, we constructed three models: the basic model (Model 1) was adjusted for age and gender. In the lifestyle model (Model 2), three lifestyle factors (regular smoking, physical exercise, and alcohol use) were added to Model 1 to investigate the potential confounding effects of lifestyles factors. In the socioeconomic model (Model 3), two socioeconomic factors (educational level and marital status) were added to Model 1 to investigate the potential confounding effects of socioeconomic factors. In the metabolic model (Model 4), blood levels of glycated hemoglobin (HbA1C) and fasting blood levels of high density lipoprotein cholesterol (HDL-c) were added to Model 2 to evaluate the potential confounding effect of metabolic status. Model 4 was constructed based on Model 2 rather than Model 3, because Model 2 showed a better goodness-of-fit ( $-2 \log$  likelihood) compared to Model 3. The potential confounders were chosen based on a known influence on the examined variables (HbA1C; HDL; (Peng et al., 2015; Zieliński and Kusy, 2015), or an observed difference between the investigated groups (regular smoking; physical exercise; alcohol use; see Table 1). To analyze the flux of the purine cycle, we formed ratio variables by dividing the level of one metabolite by the levels of its precursor (Fig. 1). A total of 6 ratio variables (i.e. inosine/adenosine, inosine/IMP, hypoxanthine/inosine, xanthine/hypoxanthine, xanthine/xanthosine, xanthine/guanine) were analyzed similarly to metabolite levels. All the above-described regression models were repeated for the metabolite ratio variables.

In the follow-up setting and in analyses between the remitted or non-remitted group and the non-depressed control group, a smaller number of participants were available for the analyses. Thus, fewer variables were utilized as covariates in the multivariate analysis in order to remain with the recommended 10% limit and avoid overfitting of the models (Babyak, 2004). Two models were used. The basic model (Model 1) was identical to Model 1 used in the baseline setting, and was adjusted for age and gender. Model 2 was further adjusted for physical exercise, as the level of physical exercise significantly differed between the analyzed groups.

The possible confounding role of medication was assessed with the Mann-Whitney  $U$  test in order to detect possible group differences in metabolite levels between MDD patients using and not using (1) any antidepressants, (2) SSRIs, (3) duloxetine, (4) venlafaxine, (5) mirtazapine, or (6) any antipsychotic. We did not examine the specific effects of bupropion and trazodone due to the

**Table 1**  
Characteristics of the study groups at baseline. Values are medians (interquartile ranges) unless otherwise stated.

	MDD n = 99	Remitted n = 33	Non-remitted n = 45	Non-depressed n = 253	Test statistics	
					MDD vs. Non-depressed	Remitted vs. Non-remitted
Age, mean (SD)	39.41 (11.94)	37.76 (12.82)	43.42 (10.13)	55.28 (10.08)	t = 12.60 <sup>a,***</sup>	t = 2.17 <sup>a</sup>
BDI	29 (19.0–36.0)	16 (7–23)	32 (20–39)	2.0 (0.0–4.0)	Z = -14.12 <sup>c,***</sup>	Z = -5.05 <sup>c,***</sup>
Female, n (%)	56 (56.6)	17 (51.5)	28 (62.2)	129 (51.0)	$\chi^2 = 0.89^b$	$\chi^2 = 0.89^b$
Married or living with a partner, n (%)	84 (84.4)	18 (54.5)	20 (44.4)	213 (84.2)	$\chi^2 = 0.02^b$	$\chi^2 = 0.78^b$
University, polytechnic or college education, n (%)	36 (36.4)	6 (18.8)	16 (35.6)	88 (34.8)	$\chi^2 = 0.08^b$	$\chi^2 = 2.59^b$
Regular smoking, n (%)	29 (29.3)	14 (42.4)	12 (26.7)	26 (10.3)	$\chi^2 = 19.52^b,***$	$\chi^2 = 2.13^b$
Significant alcohol usage, n (%)	22 (22.2)	12 (36.4)	17 (37.8)	36 (14.2)	$\chi^2 = 3.10^b$	$\chi^2 = 0.016^b$
Regular exercise, n (%)	43 (42.4)	28 (84.8)	21 (46.7)	243 (96.0)	$\chi^2 = 132.77^b,***$	$\chi^2 = 11.88^b,***$
B-HbA1C	4.9 (4.3–5.5)	–	–	5.6 (5.4–5.9)	Z = -8.02 <sup>c,***</sup>	–
fb-KolHDL	1.0 (0.7–1.5)	1.56 (1.19–1.99)	1.39 (1.15–1.83)	1.4 (1.1–1.7)	Z = -5.20 <sup>c,***</sup>	Z = -1.03 <sup>c</sup>
Diabetes, n (%)	5 (5.1)	1 (3.0)	3 (6.7)	21 (8.2)	$\chi^2 = 1.10^b$	$\chi^2 = 0.52^b$
Arterial hypertension, n (%)	32 (32.3)	6 (18.2)	19 (42.2)	78 (30.8)	$\chi^2 = 0.07^b$	$\chi^2 = 5.05^b,^*$
Asthma, n (%)	12 (12.1)	2 (6.1)	7 (15.6)	22 (8.6)	$\chi^2 = 0.95^b$	$\chi^2 = 1.68^b$

Abbreviations: MDD, major depressive disorder; SD, standard deviation.

<sup>a</sup> Student's *t*-test.

<sup>b</sup> Chi-squared test.

<sup>c</sup> Mann-Whitney *U* test.

\* *p* < 0.05.

\*\*\* *p* < 0.001.

**Table 2**  
Baseline serum levels of purine metabolites in MDD patients and non-depressed subjects, and serum levels of purine metabolites in remitters and non-remitters on follow-up. Values are medians (interquartile ranges).

		MDD n = 99	Remitted n = 33	Non-remitted n = 45	Non-depressed n = 253	Test statistics	
						MDD vs Non-depressed	Remitted vs Non-remitted
Inosine	Baseline	2.15 (1.15–4.67)	2.72 (1.23–5.23)	1.58 (0.89–3.84)	5.07 (2.57–10.75)	Z = -5.9 <sup>a,***</sup>	Z = -1.46 <sup>a</sup>
	Follow-up		9.00 (1.82–16.21)	4.825 (1.66–11.61)	–		Z = -1.46 <sup>a</sup>
Xanthine	Baseline	1.22 (0.83–1.81)	1.12 (0.77–1.61)	1.33 (0.87–1.95)	0.93 (0.64–1.41)	Z = -3.56 <sup>a,***</sup>	Z = -1.12 <sup>a</sup>
	Follow-up		1.11 (0.72–1.53)	1.10 (0.78–1.56)	–		Z = -0.23 <sup>a</sup>
Guanosine	Baseline	0.40 (0.20–0.90)	0.53 (0.2–1.15)	0.32 (0.15–0.71)	1.08 (0.61–1.78)	Z = -7.31 <sup>a,***</sup>	Z = -1.42 <sup>a</sup>
	Follow-up		1.25 (0.20–1.95)	0.79 (0.33–1.22)	–		Z = -0.68 <sup>a</sup>
Hypoxanthine	Baseline	7.06 (5.93–9.17)	7.23 (6.41–10.38)	6.71 (5.71–8.93)	7.64 (6.22–9.50)	Z = -0.75 <sup>a</sup>	Z = -1.59 <sup>a</sup>
	Follow-up		7.93 (5.53–9.21)	7.51 (5.93–9.00)	–		Z = -0.16 <sup>a</sup>
Adenosine	Baseline	0.01 (0.00–0.01)	0.01 (0–0.01)	0.01 (0–0.01)	0.01 (0.00–0.01)	Z = -3.15 <sup>a,**</sup>	Z = -0.63 <sup>a</sup>
	Follow-up		0.01 (0.01–0.03)	0.01 (0.01–0.02)	–		Z = -2.38 <sup>a,*</sup>
Xanthosine	Baseline	0.51 (0.37–0.65)	0.52 (0.4–0.66)	0.51 (0.37–0.62)	0.50 (0.37–0.74)	Z = -0.57 <sup>a</sup>	Z = -0.15 <sup>a</sup>
	Follow-up		0.46 (0.35–0.68)	0.50 (0.36–0.84)	–		Z = -0.95 <sup>a</sup>
IMP	Baseline	0.66 (0.41–0.85)	0.68 (0.34–0.93)	0.7 (0.45–0.94)	0.64 (0.41–0.92)	Z = -0.11 <sup>a</sup>	Z = -0.43 <sup>a</sup>
	Follow-up		0.56 (0.38–0.81)	0.49 (0.36–0.75)	–		Z = -0.68 <sup>a</sup>
	Change		-0.081 (-0.504–0.276)	-0.14 (-0.53–0.23)	–		Z = -0.58 <sup>a</sup>

Abbreviations; MDD, IMP, inosine 5-monophosphate.

<sup>a</sup> Mann-Whitney *U* test.

\* *p* < 0.05.

\*\* *p* < 0.01.

\*\*\* *p* < 0.001.

small group sizes. The possible confounding role of antipsychotics was further assessed by repeating the multivariate Models 1 and 2 after the exclusion of those with any antipsychotic medication (Model 3 not applied in order to avoid over-adjusting the smaller sample following the exclusions; [Babyak 2004](#)). All analyses were performed with SPSS 19.0 for Windows statistical software (SPSS Inc., Chicago, IL). Two-tailed *p*-values below 0.05 were considered to indicate statistical significance.

### 3. Results

At the time of the follow-up, according to the SCID interviews, 33 (42.3%) patients were in full or partial remission, whereas the remaining 45 (57.7%) still fulfilled the criteria for MDD. Participants of the MDD group who did not attend the follow-up assessments did not differ from those who did attend the follow-up with regards to any covariates used in the multivariate analysis (age, sex, marital



**Table 3**

Odds ratios (OR) and 95% confidence intervals (CI) for the likelihood of belonging to the major depressive disorder group vs. the non-depressed group for each one-unit increase in serum levels of each metabolite.

		OR	95% CI	p-value
Inosine	Model 1	0.85	0.79–0.91	<0.001
	Model 2	0.86	0.80–0.94	<0.001
	Model 3	0.85	0.79–0.91	<0.001
	Model 4	0.89	0.82–0.96	0.004
Xanthine	Model 1	1.64	1.09–2.45	0.017
	Model 2	1.91	1.16–3.13	0.010
	Model 3	1.66	1.11–2.49	0.014
	Model 4	2.10	1.24–3.57	0.006
Guanosine	Model 1	0.21	0.12–0.36	<0.001
	Model 2	0.26	0.15–0.48	<0.001
	Model 3	0.21	0.12–0.35	<0.001
	Model 4	0.32	0.18–0.59	<0.001
Hypoxanthine	Model 1	1.01	0.92–1.10	0.882
	Model 2	0.95	0.86–1.06	0.374
	Model 3	1.01	0.92–1.10	0.911
	Model 4	1.02	0.91–1.14	0.736
Adenosine	Model 1	745.35	0.00–2.74E8	0.312
	Model 2	134.52	0.00–1.52E11	0.645
	Model 3	878.09	0.00–3.19E8	0.299
	Model 4	4901.45	0.00–2.56E16	0.570
Xanthosine	Model 1	0.70	0.27–1.85	0.474
	Model 2	0.60	0.18–2.03	0.412
	Model 3	0.66	0.25–1.77	0.412
	Model 4	0.79	0.19–3.26	0.743
IMP	Model 1	1.20	0.54–2.67	0.657
	Model 2	1.16	0.44–3.08	0.766
	Model 3	1.18	0.53–2.63	0.688
	Model 4	1.44	0.53–3.94	0.479

Model 1: Adjusted for age and gender.

Model 2: Model 1 further adjusted for regular smoking, significant alcohol use, and regular exercise.

Model 3: Model 1 further adjusted for educational level and marital status.

Model 4: Model 2 further adjusted for B-HbA1C and high-density lipoprotein cholesterol.

Abbreviations: IMP, inosine 5-monophosphate.

status, alcohol use, smoking, regular exercise, BDI score) or any of the measured purine metabolites.

### 3.1. Setting 1: MDD patients vs. non-depressed controls

Participants with MDD were younger than the non-depressed controls. Furthermore, they were more likely to smoke and less likely to exercise regularly. The serum levels of inosine and guanosine were lower, and the levels of xanthine and adenosine were higher in participants with MDD compared to non-depressed controls (Table 2; Fig. 2). After fully adjusted logistic regression analysis (Model 4, Table 3; Fig. 2), serum levels of inosine, guanosine, and xanthine remained significantly different between the participants with MDD and the non-depressed controls. The serum levels of hypoxanthine, xanthosine, adenosine, and IMP did not significantly differ between participants with MDD and non-depressed controls. All of the ratio variables differed significantly between the MDD group and non-depressed control group after fully adjusted logistic regression analysis (Supplementary Tables 1 and 2). The correlations between the metabolites are presented in Supplementary Tables 3 and 4.

### 3.2. Setting 2: metabolites associated with MDD remission status

Non-remitted participants had higher baseline BDI scores and were less likely to exercise regularly than remitters (Table 3). At the follow-up, remitted and non-remitted participants displayed no significant differences with regard to the investigated purine metabolites (Table 2). The non-remitted group and non-depressed control group did not display any significant differences in measured purine metabolites. The remitted group had significantly

**Table 4**

Longitudinal changes in metabolite levels from baseline to follow-up within the remitted and non-remitted MDD groups.

	Non-remitted (n = 45), Z (p-value)*	Remitted (n = 33), Z (p-value)*
Inosine	−3.51 (p < 0.001)	−3.01 (p = 0.003)
Xanthine	−2.62 (p = 0.009)	−1.22 (p = 0.221)
Guanosine	−3.41 (p = 0.001)	−2.44 (p = 0.015)
Hypoxanthine	−0.05 (p = 0.959)	−0.99 (p = 0.321)
Adenosine	−1.20 (p = 0.229)	−3.10 (p = 0.002)
Xanthosine	−0.89 (p = 0.376)	−0.26 (p = 0.796)
IMP	−2.13 (p = 0.031)	−0.85 (p = 0.396)

Abbreviations: IMP, inosine 5-monophosphate.

\* Wilcoxon signed-rank test.

higher levels of adenosine compared to the non-depressed control group after logistic regression analysis adjusted for age, sex, and regular exercise (data not shown).

### 3.3. Setting 3: within-group changes in metabolite levels during the follow-up period

In the remitted group, levels of inosine, guanosine, and adenosine decreased over the follow-up period. In the non-remitted group, levels of inosine, xanthine, guanosine, and IMP decreased over the follow-up period (Table 4; Fig. 2). However, metabolite changes from the baseline to the follow-up did not correlate with changes in BDI scores (data not shown). The serum levels of xanthine, hypoxanthine, xanthosine, and IMP did not change over the follow-up period in the remitted group. Similarly, the serum levels of xanthine, hypoxanthine, and xanthosine showed no change over the follow-up period in the non-remitted group.

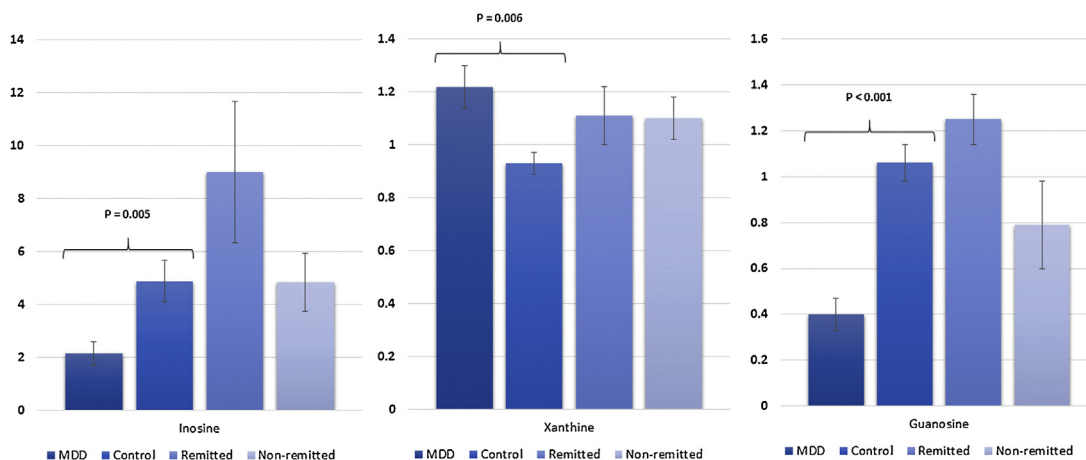
### 3.4. The effect of medication use in the MDD sample

We observed no significant differences in the serum levels of the purine metabolites between MDD patients who did not use any type of antidepressant and those who used (1) any antidepressants, (2) SSRIs, (3) duloxetine, (4) venlafaxine, (5) mirtazapine, or (6) any antipsychotic (Supplementary Tables 5–10). Furthermore, when we excluded all MDD patients using antipsychotic medication, and repeated multivariate models 1 and 2 with this subsample, our results did not change. Inosine (Model 1: OR 0.84, 95% CI 0.79–0.91, p < 0.001; Model 2: OR 0.87, 95% CI 0.80–0.96, p = 0.006), xanthine (Model 1: OR 1.81, 95% CI 1.13–3.90, p = 0.014; Model 2: OR 2.39, 95% CI 1.13–3.92, p = 0.20), and guanosine (Model 1: OR 0.19, 95% CI 0.10–0.37, p < 0.001; Model 2: OR 0.28, 95% CI 0.14–0.56, p < 0.001) remained significant after the exclusion of participants using antipsychotic medication.

## 4. Discussion

### 4.1. Summary of main findings

We observed (1) lower serum levels of inosine and guanosine, and (2) higher serum levels of xanthine in MDD patients compared with non-depressed general population controls. These findings persisted, regardless of adjustments for age, gender, alcohol use, regular smoking, physical exercise, HbA1C, and HDL-c. At the follow-up, contrary to our hypotheses, no significant differences in the changes in metabolite levels were observed between remitted vs. non-remitted groups. However, in both groups, the levels of inosine and guanosine increased during the follow-up.



**Fig. 2.** Bar chart of the metabolite levels significantly associated with belonging to the MDD group vs. the non-depressed group. Levels are given for MDD, non-depressed, remitted, and non-remitted groups. P-values after assessments with multivariate model 3 between the MDD group and non-depressed group are displayed in the figure.

#### 4.2. Comparison with previous literature

Our finding of different levels of purine cycle metabolites (specifically, inosine, guanosine, and xanthine) in depressed and healthy individuals suggests an alteration in purine metabolism in MDD. Several studies have reported low levels of uric acid, the antioxidative end product of purine metabolism, during depression (Chaudhari et al., 2010; Kesebir et al., 2014; Wen et al., 2012). These previous observations are in line with our findings suggesting altered purine metabolism that may lead to decreased levels of uric acid.

The only metabolomics study to date to compare purine metabolites in depressed and non-depressed individuals found no correlations between MDD and plasma purine metabolites (Steffens et al., 2010). However, that study focused on patients with a diagnosis of chronic heart failure, a participant group essentially different from ours, decreasing the feasibility of direct comparisons between these two studies.

Agren et al. (1983) found a correlation between lower CSF levels of purine cycle intermediates (hypoxanthine and xanthine) and depressive symptomology. In our serum samples, the hypoxanthine level was not associated with MDD. Moreover, the association between xanthine and depression was inverted in the study by Agren et al. (1983) compared to the present study. Although their study used a similar method to analyze the samples, they had a different sample type and different diagnostic criteria compared with the present study, which could explain the differences in the results.

When comparing remitted and non-remitted MDD patients, we found no significant differences in purine metabolites, consistently with a previous study focusing on CSF purine metabolites (Kaddurah-Daouk et al., 2012). However, in the whole MDD group of our study, the levels of several measured metabolites changed significantly. This suggests that either the treatment or the course of depression affects the purine cycle in a manner independent of the treatment response or severity of depression. In our study, neither the levels of purine metabolites nor changes in them were associated with the severity of symptoms (BDI score).

#### 4.3. Possible mechanisms

We found increased serum levels of xanthine and decreased serum levels of guanosine and inosine in patients with MDD. These observations suggest that circulating xanthine may accumulate in patients with MDD, possibly because of increased activation of XO and ADA (Herken et al., 2007). The accumulation of xanthine

appears to arise from increased degradation of inosine and guanosine, both of which had lower levels in the MDD group compared to the non-depressed group. Inosine is derived from IMP or adenosine, and therefore from AMP, while guanosine is derived from GMP. It is possible that intercellular levels of AMP, IMP, and GMP could be altered in depression. Indeed, during the follow-up period of the present study, the levels of IMP did not change in the remitted group, whereas in the non-remitted group the levels of IMP decreased.

Taken together, these results suggest that the purine degradation cycle may be hyperactive during depression. This increased activity of purine degradation could be due to (1) an increased need for uric acid, the anti-oxidative end product of purine metabolism, (2) an increased turnover of nucleotides to nucleosides, or (3) secondary activation of enzymes important in purine metabolism.

Even though in our samples we did not measure the levels of uric acid, which is a potent scavenger, earlier studies have demonstrated that its levels are low during depressive disorder (Chaudhari et al., 2010; Kesebir et al., 2014). As XO is a rate-limiting step in purine nucleotide catabolism (Pritsos, 2000), it appears that even though the activity of XO increases during depression, it is not enough to ensure a sufficient supply of uric acid. Therefore, the increased activity of XO (Herken et al., 2007) and the hyperactivity of the purine degradation cycle in general could be considered as a compensatory mechanism to counteract oxidative stress leading to a high demand for uric acid (Krenitsky et al., 1986). This is in line with previous evidence that depression is characterized by increased oxidative stress as a consequence, among others, of a low antioxidant capacity and higher production of reactive oxygen (ROS) and nitrogen (NOS) species (Maes et al., 2011a). In this context, the higher levels of xanthine in our sample of MDD patients compared with healthy controls, along with lower levels of guanosine and inosine, reflects a highly activated XO, possibly as a compensatory mechanism to the reduced levels of the antioxidant uric acid.

Depression is characterized by a compromised state of oxidative stress. Increased production of free reactive radicals and compromised means of defense can lead to damage to fatty acids, DNA, proteins, and mitochondria. Furthermore, the state of oxidative stress in MDD is also associated with some of the inflammatory changes in depression (Maes et al., 2011b). The purine cycle participates in the management of oxidative stress by at least two means: (1) Uric acid, the end product of the cycle, scavenges iron ions by forming complexes with them and thus eases the oxidative load of the system by decreasing lipid peroxidation (Davies et al., 1986). Lipid peroxidation is known to be especially harmful to nervous tissue, which is rich in iron (Shichiri, 2014). (2) XO, which produces

uric acid, also produces nitric oxide and superoxide radicals, both of which are oxidative compounds (Pritsos, 2000).

#### 4.4. Strengths and limitations

The large group size can be considered a strength in the context of metabolomics studies. The group size in our study, particularly with regard to the baseline comparisons between the MDD patients and the non-depressed controls, allowed us to take into account several potential confounders in multivariable analyses. We were also able to analyze the levels of several purine metabolites, which enabled us to gain a more thorough view on the role of purine cycle in depression.

Some limitations need to be taken into consideration with regard to our findings. As the MDD sample set was derived from a clinical outpatient unit, most of the participants were using antidepressant medications at the time of recruitment. Using a drug-naïve population would have provided an optimal setting for a metabolomics study. However, such approach was not viable in our recruitment setting, a university hospital tertiary care clinic. Nevertheless, we performed subgroup analyses comparing the levels of metabolites between users and non-users of antidepressants in general and of specific antidepressant categories. We observed no differences with regard to the metabolites that differed between the study groups. However, this approach might not allow optimal assessment of the true effect of the antidepressants on the measured metabolites, and separate studies are indicated to clarify these issues. Analysis of the enzymes in addition to the metabolites of the purine cycle would have further supplemented our observations. Moreover, a longer follow-up would have been beneficial to confirm our suggestion of the full normalization of purine metabolite levels during remission.

## 5. Conclusions

Our study findings suggest hyperactivity of the purine cycle in MDD patients. The levels of several purine metabolites changed significantly during the follow-up period in the MDD group. The increased activity of the purine cycle may represent a compensatory mechanism that seeks to supply a sufficient level of uric acid to counteract increased oxidative processes during depression.

## Contributors

TAS participated in the designing the study, performed statistical analyses, interpreted the data and wrote the manuscript. TT and SML designed the study and interpreted the data. ET and AR interpreted the data and participated in writing the manuscript. HV, PM, MVK and KH participated in designing the study collecting the study sample. VV performed the metabolomics analyses. All authors critically revised the manuscript for important intellectual content and have approved the final version to be published.

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## Conflict of interest

None.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2016.04.017>.

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