Neuroendocrinology Letters Volume 36 No. 5 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Higher serum concentrations of tyrosine and glutamate in schizophrenia patients treated with clozapine, compared to in those treated with conventional antipsychotics

Kristina Melkersson [1], Moira Lewitt [2], Kerstin Hall [1]

1 Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
2 School of Health, Nursing and Midwifery, University of the West of Scotland, Scotland, United Kingdom

Correspondence to: Kristina Melkersson, MD., PhD.
Department of Molecular Medicine and Surgery, Karolinska Institutet
c/o Sollentuna/ Sigtuna/ Upplands Väsby Psychiatric Polyclinic
Anton Tamms väg 3, S-194 34 Upplands Väsby, Sweden.
TEL: +46 8 12344010; FAX: +46 8 58733018; E-MAIL: Kristina.Melkersson@ki.se

Submitted: 2015-08-18 Accepted: 2015-10-08 Published online: 2015-11-29

Key words: **amino acids; tyrosine; glutamic acid; insulin; insulin resistance; schizophrenia; antipsychotic drugs; clozapine**

Neuroendocrinol Lett 2015; **36**(5):465–480 **PMID:** 26707048 NEL360515A09

Abstract

RATIONALE: The effect of long-term treatment with the atypical antipsychotic clozapine on the serum amino acid profile in schizophrenia patients has not previously been studied.
OBJECTIVES: The aim of this study was to compare serum amino acid patterns in patients on long-term clozapine treatment with long-term conventional antipsychotic treatment, and their relationships to insulin resistance and antipsychotic serum concentrations.
METHODS: Thirty-three patients with schizophrenia or schizoaffective disorder on long-term treatment (mean 8.3 years) with clozapine (n=20) or conventional antipsychotics (n=13) were studied. Amino acids were quantified in fasting serum samples by ion exchange chromatography and markers of insulin resistance and antipsychotic drug concentrations were determined by standard methods.
RESULTS: Several amino acids, most notably tyrosine and glutamic acid, were elevated above the reference range in several patients receiving clozapine. Additionally, significantly higher mean values of tyrosine (1.5-fold, p=0.001), glutamic acid (2-fold, p=0.0005) and six other amino acids were observed in the clozapine group than in the conventional antipsychotic group. Several amino acids were related to insulin resistance in both treatment groups.
CONCLUSIONS: In this study, we show that serum tyrosine and glutamic acid concentrations are markedly elevated in patients on long-term clozapine treatment, compared to patients on long-term conventional antipsychotic treatment. These findings are of importance since these two amino acids have been implicated in the pathophysiology of schizophrenia.

ORIGINAL ARTICLE

INTRODUCTION

Dysregulation of dopamine transmission in the brain and changes in glutaminergic neurotransmission are the two main hypotheses for the pathophysiology of schizophrenia (Carlsson 1978; Olney & Farber 1995). Acutely psychotic patients with schizophrenia have been shown in single photon emission computed tomography (SPECT) studies, by challenge with a low dose of amphetamine, to release more endogenous dopamine than do healthy controls or schizophrenia patients in remission (Laruelle *et al.* 1999). This indicates that acute psychotic symptoms in schizophrenia are associated with an overactive dopamine system in the brain. The glutamate hypothesis, on the other hand, is based on the observation that phenylcyclidine and ketamine, which are N-methyl-D-aspartate receptor (NMDAR) glutamate antagonists, may induce symptoms characteristic of schizophrenia in humans (Frohlich & Van Horn 2014). The precursor to dopamine formation is tyrosine (Tyr) (Wurtman *et al.* 1980) and thus two amino acids (AAs) – Tyr and glutamic acid (Glu) – are of special interest in schizophrenia. It is also well known that the metabolic syndrome occurs more often in patients with schizophrenia than in the normal population (Malhotra *et al.* 2013). Therefore, glucose-, lipid- and AA metabolism are all of interest in these patients.

In unmedicated patients with schizophrenia, the AA profiles in serum and cerebrospinal fluid (CSF) have been demonstrated to in part be changed compared to those in healthy controls (for summary of published articles see Table 1). The influence of different antipsychotics on the AA levels in serum and CSF has also previously been studied in patients with schizophrenia. No effects of conventional antipsychotics on serum levels of glycine (Gly), phenylalanine (Phe), serine (Ser), tryptophan (Trp) and Tyr or on CSF levels of Gly and Ser have been found in schizophrenia patients, when compared to healthy controls (Table 1). Neither have any effects been observed in patients with schizophrenia of three weeks of therapy with haloperidol on CSF gamma-aminobutyric acid levels, of six weeks of therapy with sulpiride on serum glutamine (Gln), Glu, Trp and Tyr levels, or of six weeks of therapy with olanzapine on CSF Glu, Gly and Ser levels (Table 1). On the other hand, four and 12 weeks of therapy with the atypical antipsychotic clozapine have been reported to slightly increase serum aspartic acid (Asp) levels and both slightly increase or decrease serum Glu levels in schizophrenia patients (Table 1). However, longer-term effects of treatment with clozapine on the serum AA profile in patients with schizophrenia have not previously been studied.

Insulin has long been known to exert an effect on AA transport across cell membranes, both on transport by System A of neutral AAs with small or unbranched sidechains as alanine (Ala), Gly and Ser and on transport by System L of large neutral amino acids (LNAAs) with branched or aromatic sidechains as several essential amino acids (EAAs) and the partly EAA Tyr, thereby influencing the serum AA pattern in man (Christensen 1990; DeFronzo & Ferrannini 1992; Kilberg *et al.* 1985; Kimball *et al.* 1994; Oxender & Christensen 1963; Wurtman *et al.* 1980; Yanagida *et al.* 2001). Today it is also well established that treatment with antipsychotics, most notably with clozapine, contributes to obesity, hyperinsulinemia and insulin resistance (IR) in patients with schizophrenia (Melkersson *et al.* 2004; Melkersson *et al.* 1999). Therefore, it would be of interest to relate body mass index (BMI), insulin levels and degree of IR to serum AAs in schizophrenia patients on therapy with different antipsychotics, particularly clozapine.

The aim of this study was therefore to analyze serum AAs in patients on long-term therapy with clozapine and compare with those in patients on long-term therapy with conventional antipsychotics, and also to examine potential relationships between serum AAs and insulin or markers of IR (Homeostasis Model Assessment of insulin resistance [HOMA-IR] and insulin-like growth factor binding protein-1 [IGFBP-1]) or insulin-like growth factor I (IGF-I), a nutritional marker. The relationships between serum AAs and antipsychotic doses and serum concentrations were also determined.

PATIENTS AND METHODS

Patients

Consecutive outpatients at psychiatric polyclinics in the region of Stockholm, Sweden, diagnosed with schizophrenia or schizoaffective disorder according to the DSM-5 criteria (American Psychiatric Association 2013) and on long-term therapy with either clozapine or conventional antipsychotics, were invited to participate in this study. Any patients having a substance-related disorder, diabetes mellitus or other physical illness that could influence the evaluation were excluded. Twenty patients receiving clozapine and 13 patients receiving conventional antipsychotics were included.

Characteristics of the patients in the two treatment groups are given in Table 2a. The majority of patients in the treatment groups were Caucasian individuals. All patients had a diagnosis of schizophrenia, except one woman in the clozapine group who had schizoaffective disorder. Most patients in both treatment groups were in full remission regarding psychotic symptoms (Table 2a). However, in the clozapine group, most patients were likely treatment-resistant to conventional antipsychotics, whereas in the conventional antipsychotic group, most patients were treatment-responsive.

The mean (SD) daily dose was 399 (147) mg of clozapine and 249 (192) mg chlorpromazine equivalents of conventional antipsychotics, calculated as previously described (Melkersson *et al.* 2001), with no significant gender differences found in daily doses within the groups (p=0.722 and p=0.700, respectively). Concomitant medications in low to moderate doses were used

Tab. 1. Summary of published articles regarding 1) AAs in serum/ plasma and/ or CSF in unmedicated patients with schizophrenia and 2) effect of different antipsychotics on AAs in serum/ plasma and/ or CSF in patients with schizophrenia, both described in chronological order.

Study design	Diagnosis, patients, controls and antipsychotics	Methods	Results	Reference
AAs in serum/ plasma and/ or CSF in unmedicated patients with schizophrenia				
Cross-sectional study, comparing the Trp serum level in psychiatric patients and healthy controls	Schizophrenia, acute or subacute psychosis (n=18) Neurosis (n=21) Controls (n=21)	FM	The Trp serum level was significantly lower in patients with schizophrenia than in those with neurosis or controls	Yayura-Tobias *et al.* 1978
Cross-sectional study, comparing 23 AAs in plasma and CSF in patients with schizophrenia and healthy controls	Schizophrenia, acute psychosis (n=37) Controls (n=65)	HPLC	Ile, Leu, Lys, Met, Phe, Val, Ala and Tau plasma levels were significantly higher in patients with schizophrenia than in controls, while His and Gln plasma levels were significantly lower The CSF/ plasma ratios did not differ for any of the 23 AAs between patients and controls	Bjerkenstedt *et al.* 1985
Cross-sectional study, comparing Gly and Ser plasma levels in patients with schizophrenia and healthy controls	Schizophrenia, psychotic phase (n=15) Controls (n=15)	GC	Gly and Ser plasma levels were significantly higher in patients with schizophrenia than in controls	Baruah *et al.* 1991
Cross-sectional study, comparing 18 CSF AAs in patients with schizophrenia or schizophreniform disorder and controls in whom internal, neurological and psychiatric disorders were excluded	Schizophrenia (n=17) or schizophreniform disorder (n=9), moderately or severely ill Controls (n=15)	HPLC	The Tau CSF concentration was significantly lower in patients than in controls, while the 17 other AAs did not differ between the groups	Do *et al.* 1995
Cross-sectional study, comparing Asp, Glu and Gly CSF concentrations in patients with schizophrenia and controls in whom psychiatric and neurological disorders were excluded	Schizophrenia (n=55) or schizoaffective disorder (n=6), only M Controls, only M (n=23)	HPLC	No significant differences were found in Asp, Glu and Gly CSF concentrations between patients and controls	Tsai *et al.* 1998
Cross-sectional study, comparing Glu and Gln levels and Gln/ Glu ratio in CSF in patients with schizophrenia and healthy controls	Schizophrenia, first-episode psychosis, only M (n=25) Controls, only M (n=17)	HPLC	The Gln/ Glu CSF ratio was significantly higher in patients than in controls, although each level of Glu and Gln did not significantly differ between the groups	Hashimoto *et al.* 2005
Cross-sectional study, comparing the Trp serum concentration in patients with schizophrenia and healthy controls	Schizophrenia (n=18) Controls (n=18)	MP	The Trp serum concentration was significantly lower in patients than in controls	Xaun *et al.* 2011
Cross-sectional study, comparing His, Arg, Glu and Orn plasma concentrations in patients with schizophrenia and healthy controls	Schizophrenia, outpatients or stable inpatients (n=52) Controls (n=216)	MP	His, Arg and Glu plasma concentrations were significantly lower in patients than in controls, while the Orn plasma concentration was significantly higher	He *et al.* 2012
Effect of different antipsychotics on AAs in serum/ plasma and/ or CSF in patients with schizophrenia				
Conventional antipsychotics				
Cross-sectional study, comparing Phe, Trp and Tyr plasma levels in neuroleptic-treated or unmedicated patients with schizophrenia and healthy controls	Schizophrenia, chronic, neuroleptic-treated (n=10) Schizophrenia, chronic, unmedicated (n=12) Controls (n=18) Standard doses of neuroleptics, otherwise NR	HPLC	No significant differences were found in Phe, Trp or Tyr plasma levels between patients and controls, or between patients receiving or not receiving neuroleptics	Potkin *et al.* 1983

Abbreviations: AAs = amino acids, CSF = cerebrospinal fluid, FM = fluorometric method according to Denckla & Dewey (1967) and as modified by Bloxam & Warren (1974), GC = gas chromatography, HPLC = high pressure liquid chromatography, IEC = ion exchange chromatography, M = men, MP = metabolomic profiling, n = number, NR = not reported, RRA = radioreceptor assay, SIDM = stable isotope dilution method

Tab. 1. *Continued*

Study design	Diagnosis, patients, controls and antipsychotics	Methods	Results	Reference
Cross-sectional study, comparing Gly and Ser plasma and CSF levels in schizophrenia patients treated with neuroleptics and healthy controls	Schizophrenia, psychotic phase (plasma samples n=56, CSF samples n=27) Controls (n=89) All patients were on therapy with neuroleptics or had been receiving neuroleptics within the last six months	IEC	No significant differences were found in Gly or Ser plasma and CSF levels between patients and controls	Perry & Hansen, 1984
Prospective study, comparing the GABA CSF concentration in patients with schizophrenia before and after three weeks of therapy with haloperidol	Schizophrenia, paranoid subtype, only M (n=19) Mean daily dose: 26±4 mg	RRA	No significant difference was found in GABA CSF concentration before and after three weeks of therapy	Gattaz *et al.* 1986
Prospective study, comparing Trp, Tyr, Glu and Gln serum levels in patients with schizophrenia before and during six weeks of therapy with sulpiride and in healthy controls	Schizophrenia, acute psychotic phase (n=24) Controls (n=10) Dose range: 400-1200 mg daily	HPLC	No significant differences were found in Trp, Tyr, Glu or Gln serum levels between patients and controls before therapy No significant changes were found between pre-treatment serum levels of Trp, Tyr, Glu and Gln and levels during six weeks of therapy	Alfredsson & Wiesel, 1989
Olanzapine				
Prospective study, comparing the Glu concentration in CSF in patients with schizophrenia before and during six weeks of therapy with olanzapine	Schizophrenia, only M (n=17) Daily dose: 10 mg	HPLC	No significant differences were found between the pre-treatment CSF concentration of Glu and concentrations during the six weeks of therapy	Scheepers *et al.* 2002
Prospective study, comparing Gly and Ser concentrations in CSF in patients with schizophrenia before and during six weeks of therapy with olanzapine	Schizophrenia, only M (n=13) Daily dose: 10 mg	SIDM	No significant differences were found between pre-treatment CSF concentrations of Gly and Ser and concentrations during the six weeks of therapy	Fuchs *et al.* 2008
Cross-sectional study, comparing the Glu plasma concentration in schizophrenia patients treated with olanzapine or clozapine at least three months and healthy controls	Schizophrenia (n=22), of whom 20 patients were receiving olanzapine and two clozapine Controls (n=20) Daily dose: olanzapine; 10-20 mg, except one patient taking 2.5 mg and another patient taking 30 mg, clozapine; within the recommended dose range, otherwise NR	MP	The Glu plasma concentration was significantly higher in the patients treated with olanzapine or clozapine than in controls	Paredes *et al.* 2014
Clozapine				
Prospective study, comparing Asp, Glu and Gly serum levels in patients with schizophrenia before and after change of therapy from conventional neuroleptics to clozapine	Schizophrenia (n=7) Mean daily dose: 393±93 mg	IEC	Asp and Glu serum levels were significantly higher in patients after four weeks of therapy with clozapine than before change of therapy, whereas the Gly serum level was unchanged	Evins *et al.* 1997
Prospective study, comparing 15 serum AAs in patients with schizophrenia before and during 12 weeks of therapy with clozapine and in healthy controls	Schizophrenia, treatment-resistant, previously conventional antipsychotic-treated (n=11) Controls (n=11) Mean daily dose: 318±130 mg	HPLC	Before start of clozapine therapy, patients had significantly higher serum levels of His, Ile, Tyr, Asp and Glu than controls, but lower serum levels of Trp, Asn and Ser. The Glu serum level significantly decreased during the course of clozapine therapy, while the other 14 AAs remained unchanged	Tortorella *et al.* 2001

Abbreviations: AAs = amino acids, CSF = cerebrospinal fluid, FM = fluorometric method according to Denckla & Dewey (1967) and as modified by Bloxam & Warren (1974), GC = gas chromatography, HPLC = high pressure liquid chromatography, IEC = ion exchange chromatography, M = men, MP = metabolomic profiling, n = number, NR = not reported, RRA = radioreceptor assay, SIDM = stable isotope dilution method

Tab. 2a. Characteristics, metabolic measurements and serum concentrations of clozapine and N-desmethylclozapine in the patients studied.

				p-values[b]		
Treatment group	**Clozapine [n=20]**	**Conventional antipsychotics [n=13][a]**	**Δ between treatment groups adjusted for gender**	**Δ between gender**	**medication* gender interaction**	**Δ between treatment groups for each gender**
Ethnicity, n	Caucasian [n=18] Asian [n=1] Hispanic [n=1]	Caucasian [n=12] Hispanic [n=1]	1.000	1.000		
Diagnosis [DSM-5]	Schizophrenia [n=19] Schizoaffective disorder [n=1]	Schizophrenia [n=13] Schizoaffective disorder [n=0]	1.000	0.455		
Clinical state regarding psychotic symptoms, n	Full remission [n=17] Partial remission [n=3]	Full remission [n=11] Partial remission [n=2]	1.000	1.000		
Men: Women, n [%]	13 [65%]: 7 [35%]	5 [38%]: 8 [62%]	0.169			
Age [year][c]	A: 45.7 (9.9) M: 44.8 (9.0) W: 47.4 (11.9)	A: 49.8 (10.3) M: 48.4 (6.4) W: 50.6 (12.5)	0.376	0.525	0.955	
Duration of disease [year][c]	A: 22.2 (9.5) M: 20.2 (8.7) W: 26.0 (10.4)	A: 24.2 (10.4) M: 20.8 (5.5) W: 26.3 (12.4)	0.903	0.125	0.960	
Duration of therapy with current antipsychotic [year][c]	A: 8.5 (3.6) M: 8.5 (4.4) W: 8.7 (1.8)	A: 7.4 (4.8)[d] M: 5.9 (5.8) W: 8.4 (4.2)[d]	0.378	0.412	0.475	
Body mass index [M ≤27, W ≤25; kg/m²][c,e,f]	A: 28 CI 26-31 M: 30 CI 26-34 W: 26 CI 23-30	A: 27 CI 24-31 M: 25 CI 21-29 W: 29 CI 23-35	0.518	0.847	0.065	
Insulin [<79; pmol/L][c,e]	A: 80 CI 59-110 M: 94 CI 62-144 W: 59 CI 37-96	A: 83 CI 47-145 M: 90 CI 22-365 W: 79 CI 39-162	0.686	0.316	0.564	
HOMA-IR[c,e]	A: 2.8 CI 2.0-3.9 M: 3.3 CI 2.1-5.2 W: 2.0 CI 1.2-3.2	A: 3.7 CI 2.0-6.8 M: 3.7 CI 0.8-16.8 W: 3.7 CI 1.7-8.1	0.250	0.407	0.427	
IGFBP-1 [M 5-75, W 15-116; µg/L][c,e,g]	A: 14 CI 10-19 M: 13 CI 9-19 W: 17 CI 9-32	A: 14 CI 11-19 M: 13 CI 6-27 W: 15 CI 11-22	0.797	0.313	0.821	
IGF-I [µg/L][c]	A: 188 (63) M: 165 (52) W: 230 (62)	A: 165 (39) M: 198 (32) W: 145 (27)	0.145	0.721	0.002	 0.192 0.002
IGF-I SD [±2SD][c,h]	A: -0.1 (1.1) M: -0.5 (0.8) W: 0.8 (1.0)	A: -0.2 (1.1) M: 0.4 (0.7) W: -0.6 (1.2)	0.471	0.676	0.002	 0.070 0.006
Serum concentrations of clozapine and N-desmethylclozapine [nmol/L][c]	A: 1424 (753), 1067 (499) M: 1492 (770), 1120 (506) W: 1299 (763), 968 (510)			0.598, 0.529		

Abbreviations: A=all, HOMA-IR=Homeostasis Model Assessment of insulin resistance, IGF-I=insulin-like growth factor I, IGFBP-1=insulin-like growth factor binding protein-1, M=men, W=women, Δ=difference.

[a]I.e. haloperidol [n=3], haloperidol + thioridazine [n=1], perphenazine [n=7] and zuclopenthixol [n=2]. [b]Bonferroni-corrected α=0.002. [c]The data are given as mean (SD) or geometric mean 95% CI. The reference range is put in square brackets. [d]1 missing value. [e]The variable was positively skewed distributed and was log-transformed before the formal analyses. [f]Reference limits according to Labhart (1986). [g]Reference range was taken from a normal population of 355 men and 240 women, aged 35-55 years, who had normal glucose tolerance (Lewitt *et al.* 2010, 2008). [h]Levels were expressed in SD scores in relation to normal reference values based on 201 men and 247 women, aged 20-96 years (Hilding *et al.* 1999).

Tab. 2b. Concomitant medication use in the patients in the two treatment groups.

Clozapine group [6/20]		**Conventional antipsychotic group** [4/13]	
Patient no.	**Type and daily dose of drug(s)**	**Patient no.**	**Type and daily dose of drug(s)**
1.	Nitrazepam, 5 mg	1.	Zopiclon, 7.5 mg
2.	Alprazolam, 0.5 mg	2.	Lithium, 168 mg
3.	Diazepam, 10 mg	3.	Orphenadrine, 100 mg; Propiomazine, 25 mg
4.	Oxazepam, 5 mg	4.	Biperiden, 2 mg; Propiomazine, 50 mg; Zopiclon, 7.5 mg
5.	Lithium, 84 mg; Zopiclon, 7.5 mg		
6.	Diazepam, 20 mg; Zopiclon, 7.5 mg		

by patients in both treatment groups (clozapine group: 6/20 (30%), conventional antipsychotic group: 4/13 (31%), Table 2b). The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden and the patients participated after giving informed consent.

Literature search

Publications concerning the 23 AAs investigated in this study were sought using the Medline database from 1966 to April 2015. Besides the names of the 23 AAs, the search terms were 'schizophrenia' and 'human' or 'antipsychotic drugs' and 'human'. Articles were in a first step reviewed for exclusion or inclusion based on title, abstract and/ or article text. Included were articles that concerned 1) AA levels in serum, plasma and/ or CSF in unmedicated patients with schizophrenia or 2) effect of antipsychotic drugs in monotherapy or in therapy with a group of antipsychotics on serum, plasma and/ or CSF AA levels in patients with schizophrenia. Excluded were articles 1) published in other languages than English, 2) in which the controls included not were healthy individuals and 3) in which the AA analysis method used was considered uncertain. In total 16 articles were selected, which together with one more article obtained from the bibliographies of articles retrieved from the Medline search, were fully reviewed and presented in Table 1.

Laboratory methods

Fasting blood samples were collected in the morning at outpatient care laboratories. After centrifugation, sera were stored at –20 °C for a maximum of 12 months before analysis. Oral antipsychotic treatment was given 12–14 h and antipsychotic depot injections 2–4 weeks before blood withdrawal.

The EAAs (histidine [His], isoleucine [Ile], leucine [Leu], lysine [Lys], methionine [Met], Phe, threonine [Thr], Trp and valine [Val]), the partly EAA Tyr and the non-essential amino acids (NEAAs) (alanine [Ala], α-aminobutyric acid [Aaba], arginine [Arg], asparagine [Asn], Asp, citrulline [Cit], Gln, Glu, Gly, ornithine [Orn], proline [Pro], Ser and taurine [Tau]) were quantified by ion exchange chromatography on a Biochrom 20 analyzer (Pharmacia, Uppsala, Sweden) as described (Jacobs 1966). Analyses were performed at the Department of Clinical Chemistry and Pharmacology, University Hospital, Uppsala, Sweden, which is certified by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC) in accordance with the international SS EN ISO/ IEC 17025 standard. The lower limit of quantification was 5 μmol/L for all AAs.

Blood glucose levels were determined by a glucose-oxidase method using the 950 Immunologic-Rate-Colorimetric system (Johnson and Johnson Clinical Diagnostics Inc., Rochester, NY, USA). Insulin was measured in serum using a commercial kit consisting of a fluoroimmunometric assay (Delfia insulin, Wallac Inc., Turku, Finland). Homeostasis Model Assessment of insulin resistance was calculated according to the formula: fasting insulin concentration (μU/mL [conversion factor: 1 pmol/L = 1/ 7.175 μU/mL]) x fasting glucose concentration (mmol/L)/ 22.5 (Matthews *et al.* 1985). Concentrations of IGFBP-1 were determined in serum according to the in-house radioimmunoassay of Povoa *et al.* (1984), slightly modified with a lower detection limit of 1.6 μg/L. The intra-assay and interassay coefficients of variation were 3% and 10%, respectively. Insulin-like growth factor I was measured in serum by an in-house radioimmunoassay designed by Bang *et al.* (1991) and expressed as age-correlated standard deviation (SD) scores based on samples from healthy men and women (Hilding *et al.* 1999). Body mass index was calculated according to the formula: BMI = body weight (kg)/ height^2 (m) (Labhart 1986). Serum concentrations of clozapine and its metabolite N-desmethylclozapine were analyzed using a high-performance liquid chromatography method as previously described (Melkersson & Dahl 2003). The lower limit of quantification was 150 nmol/L for both analytes.

Statistical methods

Data are presented as mean and SD or as geometric mean and 95% confidence interval (CI). Variables that were positively skewed distributed were log-transformed before the formal analyses. To com-

pare groups of patients on therapy with different types of antipsychotics (clozapine or conventional antipsychotics) and also control for gender, a two-way factorial analysis of variance (ANOVA) was performed. In the case of a significant medication*gender interaction, the Fisher's LSD post-hoc test was in addition employed to calculate potential differences between treatment groups for each gender. The Fisher's exact test was used to compare frequencies of variables between treatment groups, and the Student t-test was employed to compare variables within treatment groups. Linear regression analysis was used, as appropriate, to determine the relationship between variables, and ANOVA was performed to determine a potential difference between two regression lines. Pearson's correlation coefficient r was calculated between pair of variables. Forward stepwise multiple linear regression analysis was used to identify independent variables, after ensuring no violation of the assumptions of normality. To take multiple testings into account, the Bonferroni corrected α was calculated to 0.002 from 30 tests done and described in Tables 2a and 3. Otherwise, uncorrected *p* values were reported in the text, tables and figures. For multiple regression analysis a *p*-value of less than 0.01 was considered statistically significant. All calculations were made with the statistical program Statistica for Windows 10.0 (Statsoft Inc., Tulsa, OK, USA).

RESULTS

The individual AA profiles in the two treatment groups, expressed as SDs from normal range, are shown in Figure 1a–b, and in Table 3 the mean (SD) or geometric mean 95% CI of AA levels and ratios are given. Amino acids were classified as EAAs, partly EAA and NEAAs and in addition as glucogenic and/ or ketogenic AAs based on through which pathway they are metabolized in the tricarboxylic acid (TCA) cycle in the mitochondria (Table 3). As seen in Table 2a, there were more men in relation to women in the clozapine group, than in the conventional antipsychotic group, but the difference did not reach significance. No significant differences were found between the treatment groups in age, duration of disease or treatment time with current antipsychotic, after gender was taken into account (Table 2a). There was also no difference in BMI, insulin, HOMA-IR and IGFBP-1 between the treatment groups, but amongst women, IGF-I levels were lower in the conventional antipsychotic group (Table 2a). Within the clozapine group, the IGF-I SD values were significantly lower in men than in women (p=0.005). The geometric mean values of IGFBP-1 in the men (13 μg/L) and women (16 μg/L) did not differ (p=0.282), but were significantly below (p=0.0001) those in reference subjects aged 35–55 years with normal glucose tolerance (men: 20 Cl 19–21 μg/L, n=355; women: 41 Cl 39–44 μg/L, n=240; Lewitt *et al.* 2010, 2008). The IGFBP-1 levels were also inversely correlated to insulin (r=–0.53, p=0.002, n=33).

Essential amino acids

In the clozapine group, several EAAs, most notably Phe and Tyr, were elevated above the reference range (±2SD) in several patients (Figure 1a). As seen in Table 3, the EAA levels in the clozapine group, apart from Trp and the branched AAs Ile, Leu and Val, were higher or displayed a tendency of higher values than in the conventional antipsychotic group.

In both treatment groups, the Tyr levels were positively related to, or displayed a tendency towards, a positive relation to BMI, insulin and its product with glucose (HOMA-IR) or IGFBP-1 (insulin^2 ×

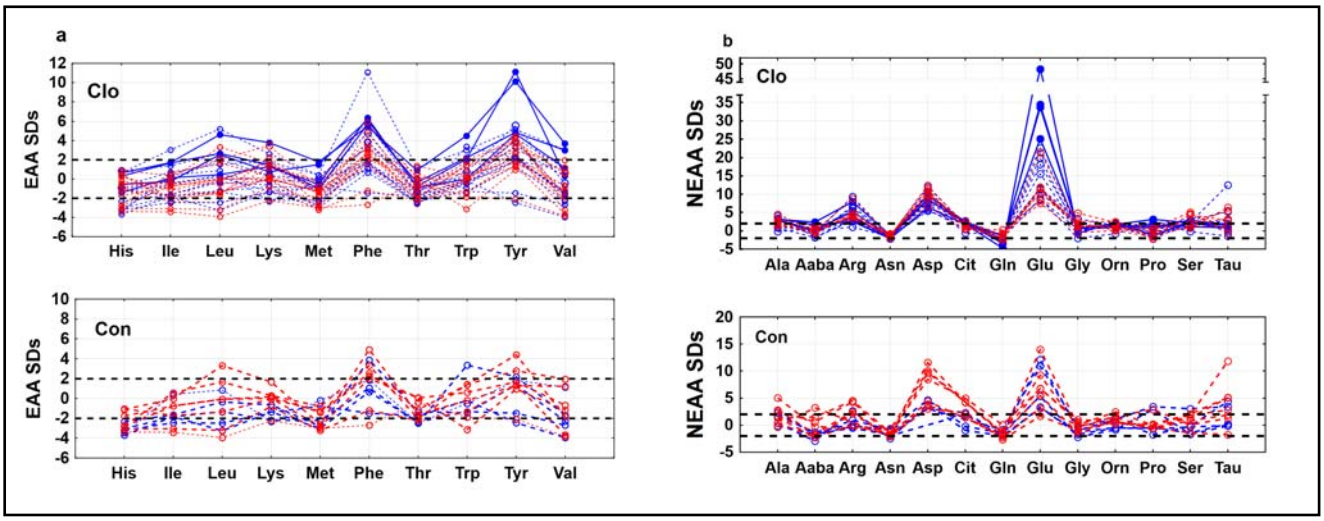

Fig. 1. a–b Amino acid profiles in serum from schizophrenia patients on long-term therapy with clozapine (Clo, n=20) or conventional antipsychotics (Con, n=13). Blue symbols represent men and red symbols women. Essential amino acid- (EAA, a) and nonessential amino acid (NEAA, b) levels are shown as the standard deviations (SDs) in relation to the normal range ±2SD (horizontal dotted lines). Values from patients with Glu/ Gln ratio >1 are connected with solid lines.

IGFBP-1; hepatic insulin resistance algorithm HIR-A) (Figure 2a–d). Together with the group difference, the variables BMI, insulin, HOMA-IR or HIR-A explained 41%, 56%, 53% and 64% respectively of the Tyr variation (p<0.0001, n=33). In the clozapine group, the regression line of Tyr on log HIR-A was shifted upwards by 62% (p<0.0001) in relation to the regression line of the other group (Figure 2d). In stepwise multiple linear regression analysis, 63% of the Tyr variation was explained by the independent variables log insulin, log IGFBP-1 and group, and neither gender, nor BMI were included as significant variables. Approximately 50% of this adjusted R^2 of 0.63 was explained by the effect of clozapine treatment.

Phenylalanine, the precursor of Tyr, displayed a pattern similar to Tyr in relation to insulin, whereas the other aromatic AA His was unrelated to insulin. In spite of being within the normal range, the mean value of His was 47% higher in the patients of the clozapine group than in the patients of the conventional antipsychotic group, in whom 10 of 13 had His values that were below normal range (Figure 1a, Table 3). There was a significant correlation between Tyr and His (r=0.66, p<0.001, n=33), without any significant difference between the equations of regression for the two treatment groups.

The sum of the branched AAs Ile, Leu, Val was positively related to insulin (r=0.64, p<0.0001, n=33). There was also a positive correlation between Tyr and the sum of the other LNAAs that use the same AA transporter (i.e. Ile, Leu, Phe, Trp and Val) (r=0.71, p<0.0001, n=33). The regression line of log Tyr on log (LNAAs – Tyr) in the clozapine group was approximately 30 µmol/L above that in the conventional antipsychotic group, but the coefficient of the slope for these linear regression lines was close to 1 only in the patients treated with conventional antipsychotics (Figure 3a).

The ratio Tyr/ (LNAAs – Tyr) tended to increase with age (r=0.38, p=0.031, n=33), but remained higher in the clozapine group than in the conventional antipsychotic group when age was taken into account (p=0.0003). In the patients receiving clozapine, there was a close significant correlation between the Tyr/ (LNAAs – Tyr) ratio and age (Figure 3b), and the addition of clozapine

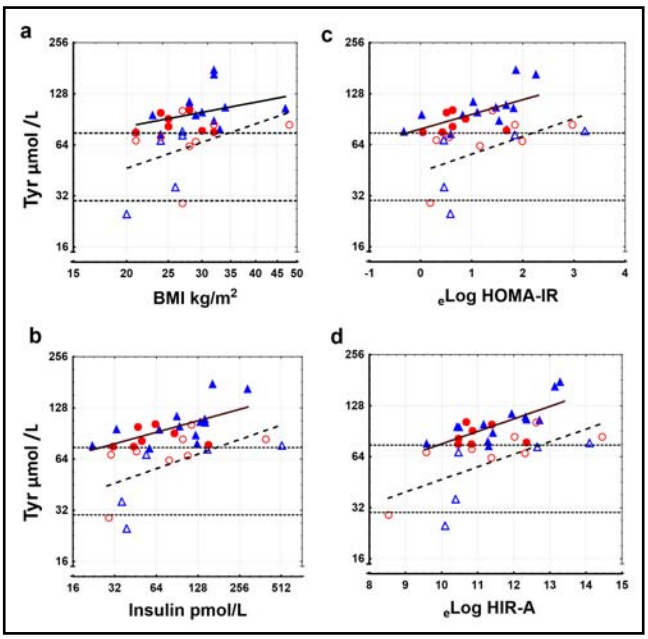

Fig. 2. a–d Tyrosine levels in relation to BMI (a), serum insulin (b), peripheral insulin resistance (HOMA-IR, c) and hepatic IR (HIR-A, d) in schizophrenia patients on long-term antipsychotic therapy. Patients treated with clozapine (n=20) are indicated by solid symbols (▲, ●) and those treated with conventional antipsychotics (n=13) by open symbols (△, ○). Blue triangles represent men and red circles women. Horizontal dotted lines indicate the reference range ±2SD of Tyr. Regression lines and correlation coefficients for the relationships:
a) $_2$log Tyr = 4.3079 + 0.4745*$_2$log BMI; r=0.38, p=0.103 (clozapine group, solid black line) significantly (p=0.0004) above $_2$log Tyr = 1.7903 + 0.8678*$_2$log BMI; r=0.43, p=0.143 (conventional antipsychotic group, dotted black line)
b) $_2$log Tyr = 5.178 + 0.2245*$_2$log insulin; r=0.62, p=0.004 (clozapine group, solid black line) significantly (p<0.0001) above $_2$log Tyr = 4.1030 + 0.2849*$_2$log insulin; r=0.61, p=0.027 (conventional antipsychotic group, dotted black line)
c) $_2$log Tyr = 6.3063 + 0.2875*$_e$log HOMA-IR; r=0.59, p=0.006 (clozapine group, solid black line) significantly (p<0.0001) above $_2$log Tyr = 5.4651 + 0.3486*$_e$log HOMA-IR; r=0.57, p=0.044 (conventional antipsychotic group, dotted black line)
d) $_2$log Tyr = 3.7954 + 0.2454*$_e$log HIR-A; r=0.71, p<0.001 (clozapine group, solid black line) significantly (p<0.0001) above $_2$log Tyr = 3.0846 + 0.2467*$_e$log HIR-A; r=0.69, p=0.009 (conventional antipsychotic group, dotted black line)

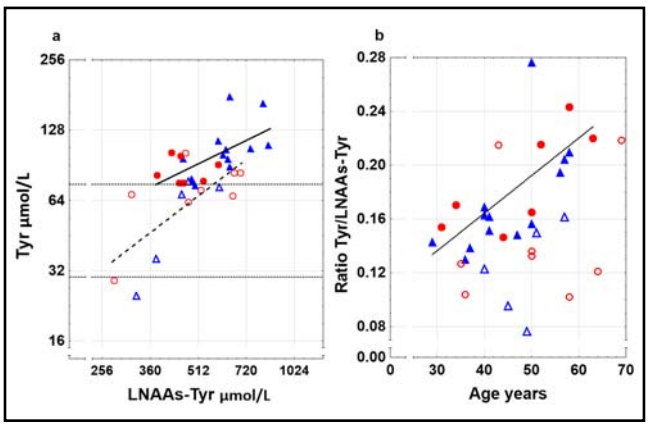

Fig. 3. a–b Relationships between Tyr and LNAAs –Tyr (a) and between ratio Tyr/ LNAAs – Tyr and age (b) in schizophrenia patients on long-term antipsychotic therapy. Blue symbols represent men and red symbols women. Patients treated with clozapine are indicated by solid symbols (▲, ●) and those treated with conventional antipsychotics by open symbols (△, ○). Horizontal dotted lines indicate the reference range ±2SD of Tyr. Regression lines and correlation coefficients for the relationships:
a) $_2$log Tyr = 0.5132 + 0.6674*$_2$log (LNAAs – Tyr); r=0.61, p=0.004 (clozapine group, n=20, solid black line) significantly (p=0.002) above $_2$log Tyr = –3.3493 + 1.0456*$_2$log (LNAAs – Tyr); r=0.72, p=0.006 (conventional antipsychotic group, n=13, dotted black line)
b) ratio Tyr/ (LNAAs – Tyr) = 0.0521 + 0.0028*age; r=0.70, p=0.001 (clozapine group, n=20, solid black line), whereas no significant relationship ratio Tyr/ (LNAAs – Tyr) = 0.0684 + 0.0013*age; r=0.33, p=0.278 (conventional antipsychotic group, n=13)

Tab. 3. Serum amino acid levels and ratios in patients on long-term treatment with clozapine or conventional antipsychotics.

Amino acids[a]	Clozapine group A:20, M:13, W:7	Conventional antipsychotic group A:13, M:5, W:8	*p*-values[b]			
			Δ between treatment groups adjusted for gender	Δ between gender	medication* gender interaction	Δ between treatment groups for each gender
Essential						
Histidine, His [65-115] µmol/L	A: 85 (12) M: 83 (15) W: 88 (7)	A: 58 (11) M: 51 (7) W: 62 (11)	**<0.00001**	0.081	0.469	
Isoleucine, Ile [40-85] µmol/L	A: 59 (16) M: 66 (15) W: 47 (9)	A: 50 (18) M: 46 (12) W: 53 (21)	0.220	0.282	0.032	0.020 0.478
Leucine, Leu [100-160] µmol/L	A: 141 (31) M: 156 (28) W: 113 (13)	A: 121 (34) M: 110 (25) W: 127 (38)	0.130	0.214	0.007	0.004 0.354
Lysine, Lys [120-220] µmol/L	A: 197 (36) M: 201 (38) W: 188 (32)	A: 157 (36) M: 131 (17) W: 174 (35)	0.002	0.237	0.032	**0.0004** 0.422
Methionine, Met [15-35] µmol/L	A: 22 (6) M: 23 (6) W: 18 (5)	A: 16 (6) M: 16 (6) W: 16 (6)	0.032	0.228	0.260	
Phenylalanine, Phe [40-70] µmol/L	A: 84 (19) M: 88 (21) W: 76 (12)	A: 69 (21) M: 64 (15) W: 72 (24)	0.062	0.766	0.151	
Threonine, Thr [90-195] µmol/L	A: 123 (31) M: 123 (26) W: 122 (41)	A: 105 (24) M: 88 (13) W: 116 (23)	0.055	0.182	0.159	
Tryptophan, Trp [30-65] µmol/L	A: 54 (13) M: 58 (14) W: 47 (8)	A: 46 (15) M: 47 (17) W: 45 (15)	0.260	0.207	0.376	
Tyrosine, Tyr[c] [30-75] µmol/L	A: 100 (28) M: 107 (32) W: 86 (11)	A: 65 (23) M: 56 (24) W: 71 (21)	**0.001**	0.765	0.061	
Valine, Val [175-300] µmol/L	A: 230 (61) M: 254 (57) W: 186 (41)	A: 199 (65) M: 178 (59) W: 211 (69)	0.243	0.414	0.025	0.018 0.408
Tyr/ (LNAAs -Tyr) ratio	A: 0.18 (0.04) M: 0.17 (0.04) W: 0.19 (0.04)	A: 0.14 (0.04) M: 0.12 (0.04) W: 0.14 (0.05)	0.004	0.210	0.780	
Trp/ (LNAAs -Trp) ratio	A: 0.09 (0.02) M: 0.09 (0.02) W: 0.09 (0.02)	A: 0.10 (0.03) M: 0.11 (0.04) W: 0.09 (0.03)	0.321	0.414	0.132	
Non-essential						
Alanine, Ala [185-455] µmol/L	A: 474 (76) M: 467 (82) W: 486 (69)	A: 446 (111) M: 407 (74) W: 470 (127)	0.270	0.240	0.519	
α-aminobutyric acid, Aaba [10-40] µmol/L	A: 25 (8) M: 25 (9) W: 26 (5)	A: 19 (13) M: 11 (5) W: 23 (14)	0.032	0.075	0.134	
Arginine, Arg [30-125] µmol/L	A: 174 (42) M: 173 (46) W: 177 (34)	A: 119 (33)[d] M: 99 (24) W: 136 (31)[d]	**0.0006**	0.177	0.268	

Abbreviations: A=all; LNAAs=large neutral amino acids; M=men; W=women; Δ=difference.
[a]Amino acids classified as glucogenic and/ or ketogenic according to Koolman & Roehm (2013) are underlined.The levels are given as mean (SD) or geometric mean 95% CI. The reference ranges that are put in square brackets are based on values from healthy individuals living in the middle of Sweden and are the ones provided by the routine laboratory. [b]Bonferroni-corrected α=0.002. *P*-values <0.002 are written in bold text. [c]Partly essential amino acid according to Koolman & Roehm (2013). [d]2 missing values. [e]1 missing value. [f]The variable was positively skewed distributed and was log-transformed before the formal analyses. [g]The higher Glu level in the clozapine-treated patients than in the conventional antipsychotic-treated patients remained after exclusion of the five patients in partial remission regarding psychotic symptoms (233 CI 183-296 vs 125 CI 95-164, *p*=0.0036).

Tab. 3. *Continued*

Amino acids[a]	Clozapine group A:20, M:13, W:7	Conventional antipsychotic group A:13, M:5, W:8	*p*-values[b]			
			Δ between treatment groups adjusted for gender	Δ between gender	medication* gender interaction	Δ between treatment groups for each gender
Asparagine, Asn [20-100] µmol/L	A: 29 (8) M: 26 (6) W: 35 (8)	A: 28 (11) M: 29 (15) W: 27 (9)	0.398	0.335	0.109	
Aspartic acid, Asp [0-15] µmol/L	A: 41 (8) M: 38 (7) W: 45 (7)	A: 31 (13)[e] M: 21 (3)[e] W: 36 (13)	**0.0007**	0.003	0.210	
Citrulline, Cit [15-50] µmol/L	A: 47 (8) M: 48 (8) W: 44 (6)	A: 52 (19)[d] M: 40 (14)[e] W: 58 (18)[e]	0.549	0.113	0.017	 0.195 0.029
Glutamic acid, Glu[f,g] [10-60] µmol/L	A: 228 CI 184-283 M: 264 CI 198-352 W: 174 CI 135-225	A: 115 CI 90-149 M: 113 CI 66-195 W: 117 CI 81-168	**0.0005**	0.230	0.167	
Glutamine, Gln [355-725] µmol/L	A: 380 (100) M: 360 (105) W: 417 (85)	A: 388 (64) M: 387 (50) W: 389 (75)	0.978	0.375	0.408	
Glycine, Gly[f] [140-340] µmol/L	A: 280 CI 247-316 M: 258 CI 223-298 W: 325 CI 260-407	A: 205 CI 178-235 M: 173 CI 132-226 W: 228 CI 196-264	**0.00008**	0.005	0.799	
Ornithine, Orn [20-105] µmol/L	A: 89 (17) M: 89 (16) W: 88 (19)	A: 76 (20) M: 64 (20) W: 83 (17)	0.031	0.179	0.128	
Proline, Pro [95-270] µmol/L	A: 187 (66) M: 201 (69) W: 162 (55)	A: 203 (75)[e] M: 212 (102) W: 196 (57)[e]	0.393	0.303	0.671	
Serine, Ser [75-170] µmol/L	A: 177 (30) M: 169 (20) W: 191 (41)	A: 133 (36) M: 120 (45) W: 141 (30)	**0.0002**	0.078	0.980	
Taurine, Tau[f] [30-80] µmol/L	A: 80 CI 67-96 M: 78 CI 61-100 W: 84 CI 60-119	A: 83 CI 62-109 M: 71 CI 46-110 W: 91 CI 59-141	0.946	0.312	0.588	
Glu/ Gln ratio[f]	A: 0.63 CI 0.44-0.90 M: 0.78 CI 0.47-1.29 W: 0.42 CI 0.28-0.63	A: 0.30 CI 0.24-0.37 M: 0.29 CI 0.19-0.46 W: 0.30 CI 0.22-0.42	0.008	0.221	0.173	

Abbreviations: A=all; LNAAs=large neutral amino acids; M=men; W=women; Δ=difference.
[a]Amino acids classified as glucogenic and/ or ketogenic according to Koolman & Roehm (2013) are underlined.The levels are given as mean (SD) or geometric mean 95% CI. The reference ranges that are put in square brackets are based on values from healthy individuals living in the middle of Sweden and are the ones provided by the routine laboratory. [b]Bonferroni-corrected α=0.002. *P*-values <0.002 are written in bold text. [c]Partly essential amino acid according to Koolman & Roehm (2013). [d]2 missing values. [e]1 missing value. [f]The variable was positively skewed distributed and was log-transformed before the formal analyses. [g]The higher Glu level in the clozapine-treated patients than in the conventional antipsychotic-treated patients remained after exclusion of the five patients in partial remission regarding psychotic symptoms (233 CI 183-296 vs 125 CI 95-164, *p*=0.0036).

serum concentration increased adjusted R^2 from 0.46 to 0.53 (p=0.073).

However, there were no relationships found between Tyr or the other EAAs and the doses of antipsychotics in the two treatment groups (data not shown).

Nonessential amino acids

The levels of the NEAAs Arg, Asp, Glu, Gly and Ser were significantly higher in the clozapine group than in the conventional antipsychotic group (Table 3). For Arg and Ser, also the number of patients having levels above the upper reference limit was greater among those receiving clozapine than among the others (Figure 1b: Arg 19/20 vs 5/11, Ser 13/20 vs 2/13).

The most striking finding in NEAA pattern was the highly elevated Glu level with about twofold higher geometric mean (98%, p=0.0002) in the clozapine group than in the conventional antipsychotic group (Table 3: 228 Cl 184–283 µmol/L vs 115 Cl 90–149 µmol/L, Figure 1b). Four men in the clozapine group reached Glu values higher than their Gln values and had Glu/ Gln ratios above 1. The Glu/ Gln ratio was also higher in the clozapine group than in the conventional antipsychotic group (Table 3). In stepwise multiple linear regression analysis, the variables BMI, insulin, HOMA-IR or HIR-A were included as positive variables together with the group variable and explained 51%, 45%, 46% and 50% respectively of the Glu variation (p<0.0001, n=33) (Figure 4a). The Gln values did not differ between the treatment groups. However in the clozapine group, but not in the conventional antipsychotic group, the Gln levels were inversely related to insulin or HOMA-IR (r=–0.62 or r=–0.63, p<0.003). As seen in Figure 4b, there was a close inverse correlation between Glu and Gln in the clozapine group, whereas a

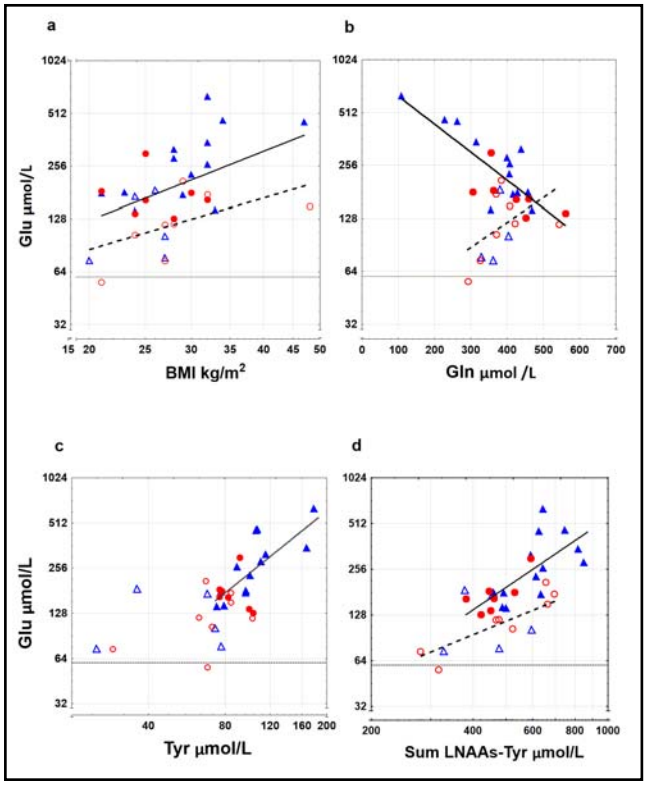

Fig. 4. a–d Glutamic acid levels in relation to BMI (a), Gln (b), Tyr (c) and to the other LNAAs transported by the same AA transporter as Tyr (d) in schizophrenia patients on long-term antipsychotic therapy. Patients treated with clozapine (n=20) are indicated by solid symbols (▲, ●) and those treated with conventional antipsychotics (n=13) are indicated by open symbols (△, ○). Blue triangles represent men and red circles women. Horizontal dotted line indicates the upper reference limit +2SD of Glu. Regression lines and correlation coefficients for the relationships:
a) $_2$log tGlu = 1.5236 + 4.3415*$_e$log BMI, r=0.54, p=0.014 (clozapine group, solid black line) significantly (p<0.0001) above $_2$log tGlu = 2.2280 + 3.2266*$_e$log BMI, r=0.50, p=0.085 (conventional antipsychotic group, dotted black line)
b) $_2$log tGlu = 9.8407 – 0.0053*Gln; r=–0.80, p<0.0001 (clozapine group, solid black line) in contrast to $_2$log tGlu = 5.0427 + 0.0047*Gln; r=0.49, p=0.087 (conventional antipsychotic group, dotted black line)
c) $_2$log tGlu = 1.2094 + 4.5521*$_e$log Tyr; r=0.72, p<0.001 (clozapine group, solid black line), whereas no significant relationship $_2$log tGlu = 5.2587 + 0.8927*$_e$log Tyr; r=0.28, p=0.360 (conventional antipsychotic group)
d) $_2$log tGlu = –5.9750 + 5.0305*$_e$log (LNAAs – Tyr); r=0.73, p<0.001 (clozapine group, solid black line) significantly (p=0.0007) above $_2$log tGlu = –1.3156 + 3.0305*$_e$log (LNAAs – Tyr); r=0.65, p=0.016 (conventional antipsychotic group, dotted black line)

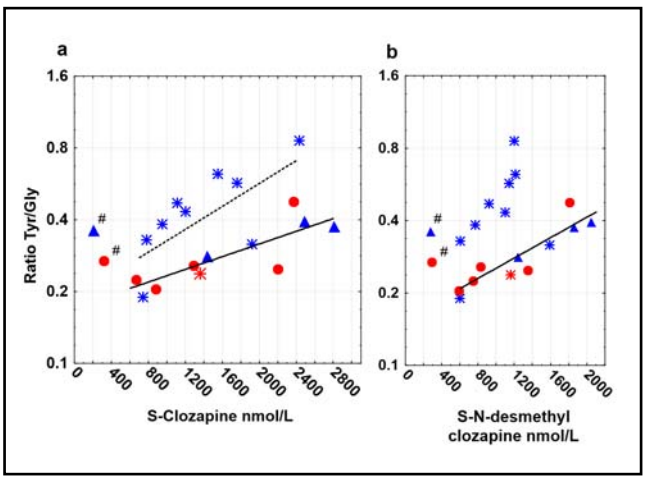

Fig. 5. a–b Ratio Tyr/ Gly in relation to serum concentrations of clozapine (a) and N-desmethylclozapine (b) in schizophrenia patients on long-term treatment. Blue symbols represent men and red symbols women. Patients with IGF-I SD <0 are indicated by stars and those with IGF-I SD >0 by solid triangles and circles. Regression lines and correlation coefficients for the relationships:
a) $_2$log ratio (Tyr/ Gly)*10 = 0.9109 + 0.7970x10^{-3}*s-clozapine concentration; r=0.66, p=0.039 (IGF-I SD <0, n=10, dotted black line) significantly (p=0.007) above $_2$log ratio (Tyr/ Gly)*10 = 0.7791 + 0.4420x10^{-3}*s-clozapine concentration; r=0.82, p=0.013 (IGF-I SD >0, n=8, two patients with low clozapine doses (100 and 200 mg daily) excluded [#], solid black line)
b) $_2$log ratio (Tyr/ Gly)*10 = 1.2230 + 0.7660x10^{-3}*s-N-desmethylclozapine concentration; r=0.36, p=0.307 (IGF-I SD <0, n=10), and a significant relationship $_2$log ratio (Tyr/ Gly)*10 = 0.6377 + 0.7040x10^{-3}*s-N-desmethylclozapine concentration; r=0.91, p=0.002 (IGF-I SD >0, n=8, two patients with low clozapine doses (100 and 200 mg daily) excluded [#], solid black line)

tendency of opposite positive correlation was observed in the conventional antipsychotic group. Levels of Glu were positively related to Tyr and Phe only in the clozapine group, and to the sum of the LNAAs except Tyr and of all EAAs apart from Thr in both groups (Figure 4c–d).

The relation between Asp and Asn was similar to that seen for the Glu and Gln relation. Aspartic acid was significantly higher in the clozapine group than in the conventional antipsychotic group, whereas Asn did not differ between the groups (Table 3). The Arg values which, similar to His, lacked relation to insulin and IR, were 46% higher in the clozapine group than in the conventional antipsychotic group and correlated with His values (r=0.81, $p<0.0001$, n=33). Besides Gln and Asn, the NEAAs which did not differ significantly between the two treatment groups were Ala, Aaba, Cit, Orn, Pro and Tau. In both groups, Ala concentrations were at upper range of normal reference values.

Glycine and Ser, which were closely correlated (r=0.87, $p<0.0001$, n=33), were both significantly higher in the clozapine group with means at upper level of the reference range. In the clozapine group, Gly tended to be inversely related to insulin and HOMA-IR and positively related to IGFBP-1 (r=–0.51, –0.52 and 0.51, $p=0.02$ respectively). With Ser as dependent variable, there was a significant correlation with IGFBP-1 (r=0.67, $p<0.001$, n=20). The ratio Tyr/ Gly, which did not differ between the two treatment groups, were positively correlated to log insulin (r=0.66, $R^2=0.44$, $p<0.0001$, n=33), without any significant influence of gender, age, treatment group or IGF-I SD.

Glycine displayed a tendency to inverse relationships to the serum concentrations of clozapine and N-desmethylclozapine (r=–0.45, $p=0.047$ and r=–0.40, $p=0.083$, respectively), whereas there was a tendency to a positive relationship between the log Tyr/ Gly ratio and the clozapine serum concentration (r=0.45, $p=0.047$). A clearly significant association was reached between the log Tyr/ Gly ratio and the clozapine serum concentration (r=0.73, $p=0.003$, n=18), when IGF-I SD above or below zero was included as independent variables in the calculation, but it required exclusion of two patients on low clozapine doses (100 and 200 mg daily). With these corrections, the association between the log Tyr/ Gly ratio and the N-desmethylclozapine serum concentration also improved (r=0.64, $p=0.019$, n=18). As seen in Figure 5a and b, the regression line of Tyr/ Gly ratio on clozapine serum concentration in patients with IGF-I SD below zero was significantly elevated ($p=0.007$) in relation to in those with IGF-I SD above zero. In patients with IGF-I SD above zero, clozapine levels explained 67% ($p=0.013$, n=8) of the Tyr/ Gly variation and its metabolite 83% ($p=0.002$, n=8). In those with IGF-I SD below zero, clozapine levels explained 43% ($p=0.039$, n=10) of the Tyr/ Gly variation, whereas its metabolite did not reach significance.

Of the other NEAAs, Gln displayed a tendency to inverse relationship to the serum concentration of clozapine, but not of N-desmethylclozapine (r=–0.41, $p=0.070$ and r=–0.18, $p=0.442$, respectively). However, no relationships were found between the NEAAs or the Glu/ Gln ratio and the antipsychotic doses in the two treatment groups (data not shown).

DISCUSSION

In this study we show that in schizophrenia patients on long-term antipsychotic treatment, particularly in those on clozapine, Glu concentrations are markedly elevated and accompanied by elevated levels of Tyr and Phe as well as of several other glucogenic and/ or ketogenic EAAs and NEAAs. Obesity and associated IR may play a role in the elevated concentrations of these AAs in both clozapine- and conventional antipsychotic-treated patients. In addition, clozapine may have a significant independent own effect on these AA levels.

The close associations found between the concentrations of Glu and Tyr and several other glucogenic and/ or ketogenic AAs, especially in the clozapine group, suggest a mitochondrial disturbance with reduction of the entrance of Glu, Tyr and other glucogenic and/ or ketogenic AAs into the TCA cycle. The elevated AAS may have been primarily affected, or secondarily influenced, for example His and Arg, which both are precursors to Glu in the TCA cycle (Koolman & Roehm 2013), and therefore may have been increased secondary to the Glu elevation, without any direct relation to insulin and IR. However, in the present study we have not determined the products of AA metabolism such as α-ketoglutarate, malate and oxaloacetate and therefore the activity of different enzymes in the TCA cycle are unknown.

In our search in the Medline database, we have found two published studies in which the AA pattern has been investigated in schizophrenia patients on short-term therapy with clozapine (Evins *et al.* 1997; Tortorella *et al.* 2001), but no studies in patients on long-term therapy. After short-term therapy, both slightly elevated and reduced Glu serum levels have been reported (Evins *et al.* 1997; Tortorella *et al.* 2001). In in vitro studies, clozapine has been shown to cause oxidation of mitochondrial proteins involved in energy metabolism, including mitochondrial malate dehydrogenase (Baig *et al.* 2010; Walss-Bass *et al.* 2008). Recently, the research group of Walss-Bass also performed metabolomic profiling in plasma of 60 schizophrenia patients on at least three month therapy with different second generation antipsychotics, and compared them with 20 healthy controls (Paredes *et al.* 2014). They reported significantly elevated concentrations of Glu and decreased levels of 2-hydroxyglutarate in the subgroup of patients who were treated with clozapine (n=2) or the structurally similar agent olanzapine (n=20). They proposed a causal relation between reduced activity of enzymes

in the TCA cycle during therapy with these two antipsychotics and the shift towards lipogenesis and risk of metabolic syndrome and decreased use of AAs in energy metabolism. Since Glu enters the mitochondria and converts to α-ketoglutarate in the TCA cycle, that in turn is the precursor of 2-hydroxyglutarate (Struys 2006), these findings support the view that clozapine and structurally similar antipsychotics reduce entrance and metabolism of Glu in mitochondria.

It seems unlikely that the high Glu levels in our patients are due to leakage from erythrocytes (Alfredsson *et al.* 1988), since all blood samples in the study were centrifuged soon after sampling and stored as serum. No clinical signs of kidney or liver disease were observed even in the four men on clozapine therapy with Glu/ Gln ratio above 1. None of these men were on drugs other than clozapine; neither did they differ in psychiatric phenotype from the other patients. Three of these four men were also in full remission with regard to psychotic symptoms. However, the man with the highest Glu level (639 μmol/L), who died at approximately 60 years of age of unknown cause, had a previous history of substance abuse.

In our patients receiving clozapine, the inverse relation between Glu and Gln serum levels, together with the markedly elevated Glu levels, the increased Glu/ Gln ratio and the tendency towards decreasing Gln levels with increasing clozapine serum concentration, raise the suspicion that clozapine may inhibit the enzyme glutamine synthetase (GS). In the brain, released Glu into the synaptic spatium is taken up and formed to Gln by GS in glia cells, which in turn supply the presynaptic neurons with Gln for their neurotransmitter production of Glu (Koolman & Roehm 2013). In post-mortem brains from patients with schizophrenia, the GS expression has been found to be higher in thalamus, but lower or unchanged in prefrontal cortex, compared to controls (Bruneau *et al.* 2005; Burbaeva *et al.* 2003; Gluck *et al.* 2002). In the liver, GS expression is nutrition-dependent and localized to the acinus (Brosnan & Brosnan 2009). In both brain and liver, the effect of clozapine on GS expression is unknown. Besides possible effect of clozapine on the GS expression and/ or on the entrance of Glu into mitochondria, clozapine might affect the transport of Glu and/ or Gln across cell membranes (Bruneau *et al.* 2005; Pineda *et al.* 1999; Varoqui *et al.* 2000; Yanagida *et al.* 2001). In the pathophysiology of schizophrenia, it has been suggested that NMDAR hypofunction is involved and that this leads to disturbed glutaminergic neurotransmission (Frohlich & Van Horn 2014; Olney & Farber 1995). In drug-naïve or minimally-medicated patients with first episode schizophrenia, both increased Gln/ Glu (= decreased Glu/ Gln) ratio in CSF and increased Gln level and Gln/Glu ratio in specific brain areas measured by proton magnetic resonance spectroscopy (^{1}H-MRS), have been reported (Bustillo *et al.* 2010; Hashimoto *et al.* 2005; Théberge *et al.* 2002, 2007). Hence, it follows that clozapine treatment, by increasing the Glu level and Glu/ Gln ratio, may counteract the aberrant Glu-Gln balance in the brain that is suggested to be a primary change in schizophrenia. Recently, Egerton *et al.* (2012) showed in a ^{1}H-MRS study that non-remitted first episode schizophrenia patients had significantly higher levels of Glu in the anterior cingulate cortex than those in remission. Most of their patients were however on treatment with different second or third generation antipsychotics (except clozapine) (Egerton *et al.* 2012). In contrast, in unmedicated patients with schizophrenia or schizoaffective disorder, the Glu concentration in CSF has been shown to decrease with increasing degree of psychotic symptoms (Faustman *et al.* 1999). In this study on serum Glu levels in patients treated with clozapine or conventional antipsychotics, most patients in both treatment groups were in full remission with regard to psychotic symptoms, and the higher Glu level in the clozapine-treated patients compared with the conventional antipsychotic-treated patients remained after exclusion of the few patients in partial remission. However, a link between peripheral Glu levels and brain levels has not yet clearly been established, and it would be of interest to further validate this link by ^{1}H-MRS of specific brain areas together with serum (and CSF) analyses of Glu (and of other AAs such as Gln and Tyr as well).

The elevated serum levels of Tyr, which were associated with Glu elevation and found particularly in our clozapine-treated patients, are of special interest, since Tyr is the precursor to dopamine formation in the brain (Wurtman *et al.* 1980). The dopamine hypothesis, postulating a dysregulation of dopamine transmission in the brain, remains the main theory for the pathophysiology of schizophrenia (Carlsson 1978; Seeman & Seeman 2014). Additionally, the striatal dopamine$_2$ receptor occupancy in the brain is significantly lower during treatment with clozapine (20%–67%) than with conventional antipsychotics (70%–90%), supporting the view that clozapine acts by a different antipsychotic mechanism from that of conventional antipsychotics (Farde *et al.* 1989).

Patients with the genetic tyrosinemias with equally elevated serum levels of Tyr have distinct clinical symptoms (Scott 2006). This absence of clinical symptoms at high serum levels of Tyr in our patients receiving clozapine suggests that reduction of Tyr entrance into the TCA cycle in the mitochondria in cells is not the sole explanation of the high Tyr serum levels, and that it may be that clozapine also affects the AA transporter and reduces the Tyr uptake into cells. System L that is responsible for the transport of LNAAs with branched or aromatic sidechains as Tyr and several EAAs across cell membranes (Kanai *et al.* 1998; Vumma *et al.* 2008; Yanagida *et al.* 2001) may be affected.

Tyrosine that is in part an EAA is formed from Phe in the liver and to a limited extent in the brain (Wurt-

man *et al.* 1980). The Tyr concentration in the brain is therefore dependent on the transport of Tyr across the blood-brain-barrier and neuronal membranes, which takes place in competition with the other LNAAs (i.e. Leu, Ile, Phe, Trp and Val) (Choi & Pardridge 1986; Wurtman *et al.* 1980). In the present study, the Tyr levels in both treatment groups were positively related to the other LNAAs, which are using the same transporter, as well as to IR and BMI. In the patients receiving conventional antipsychotics, the coefficient of the slope of the regression line of Tyr on LNAAs – Tyr was close to 1, indicating proportional changes which are expected; if the increase of AA levels in serum mainly were due to attenuation of a common transporter. The disproportional changes between Tyr and LNAAs – Tyr and the elevated regression line of Tyr on IR in our patients receiving clozapine indicate an additional mechanism, and the Tyr levels in the clozapine group were also closely associated with Glu levels. Interestingly, the Tyr/ Gly ratio positively increased with increasing serum concentrations of clozapine and its metabolite, in an IGF-I dependent manner. However, to what extent the high Tyr concentrations in the circulation of our schizophrenia patients on clozapine therapy reflect their Tyr pattern in the CSF and brain remains unknown. Previous positron emission tomography studies have shown lower Tyr influx rate over the blood-brain-barrier as well as different regulation of the Tyr influx in schizophrenia patients (Wiesel *et al.* 1999, 1991) and, in fibroblasts from schizophrenia patients, the transport of Tyr across cell membranes is aberrant (Flyckt *et al.* 2001; Hagenfeldt *et al.* 1987; Ramchand *et al.* 1996). In immediate support of our present Tyr findings, earlier *in vitro* studies have shown that clozapine (in 10^{-4} M for 1 min) significantly lower the Tyr uptake in fibroblasts from schizophrenia patients, in contrast to conventional antipsychotics (in 10^{-6}, 10^{-4}, 10^{-3} or 1 M for 1 min) that do not affect the uptake (Bongiovanni *et al.* 2013; Wiesel *et al.* 1994).

There has also been an interest in endogenous agonists that can restore a potential NMDAR hypofunction in schizophrenia, such as Gly which is an extrasynaptic NMDAR co-agonist and D-Ser which is a synaptic NMDAR co-agonist (Papouin *et al.* 2012). The NEAAs Gly and Ser have therefore been tested as adjuncts to antipsychotics for improving the treatment of schizophrenia. Administration of Gly or Ser resulted in improvements in the schizophrenia illness when they were added to conventional antipsychotics (Heresco-Levy *et al.* 1996; Goff *et al.* 1999), but not when they were added to clozapine (Potkin *et al.* 1999; Tsai *et al.* 1999). The findings in our clozapine-treated patients of a tendency towards decreasing serum levels of Gly or Ser with increasing insulin/ decreasing IGFBP-1, together with a tendency towards decreasing serum levels of Gly with increasing clozapine and N-desmethylclozapine serum concentrations, suggest that clozapine itself may have an effect on the transport across cell membranes of Gly and Ser, which both mainly are transported by the insulin-regulated system A (DeFronzo & Ferrannini 1992; Kilberg *et al.* 1985; Kimball *et al.* 1994). This might also explain the lack of improvement in the schizophrenia treatment by Gly or Ser when combined with clozapine.

Limitations of this study include its small sample size, which may limit the generalizability of our findings and require further studies with larger number of patients, and the fact that most of our clozapine-treated patients were likely to be treatment-resistant to conventional antipsychotics while most conventional antipsychotic-treated patients were not (Demjaha *et al.* 2012; Roberts *et al.* 2009). It is therefore difficult to be certain whether the findings are a consequence of clozapine, the treatment-resistant state, or both. This may explain the lack of difference in Trp level between our treatment groups. Plasma Trp levels have earlier been found to be lower in patients with treatment-resistant schizophrenia (Lee *et al.* 2011). Strengths of the study, on the other hand, include that the patients of both treatment groups had been receiving each type of antipsychotic on average 8 years before the study start and that the two treatment groups also were fully comparable regarding age, ethnicity, main diagnosis, disease duration, BMI and markers of IR (i.e. insulin, IGFBP-1, HOMA-IR and HIR-A). Furthermore, the use of concomitant medications and patients' full or partial remission regarding psychotic symptoms were comparable between the two treatment groups. Previous studies have shown greater effects of clozapine than of other antipsychotics on metabolic variables including IR measures (i.e. insulin, HOMA-IR, oral glucose tolerance and intravenous glucose tolerance) (Henderson *et al.* 2009; Howes *et al.* 2004; Rettenbacher *et al.* 2007). This absence of differences in IR measures in our present study may be explained by the fact that BMI was similar between the two treatment groups and that patients with diabetes mellitus were excluded. Our finding that 44% of the variation of insulin was explained by the ratio Tyr/ Gly raises the question how changes of the AA metabolism influences the IR, even if we at present do not know the causal relation.

In conclusion, we show that serum Tyr and Glu concentrations in particular are markedly elevated in patients on long-term treatment with clozapine, compared with in those on long-term treatment with conventional antipsychotics. These findings are of importance since these two AAs are hypothesized to be implicated in the pathophysiology of schizophrenia.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Research Council (K.M.) and Bert von Kantzows foundation (K.H.).

REFERENCES

1 Alfredsson G, Wiesel F-A (1989). Monoamine metabolites and amino acids in serum from schizophrenic patients before and during sulpiride treatment. Psychopharmacology. **99**: 322–327.
2 Alfredsson G, Wiesel F-A, Lindberg M (1988). Glutamate and glutamine in cerebrospinal fluid and serum from healthy volunteers – analytical aspects. J Chromatogr. **424**: 378–384.
3 American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders, 5th edition. Arlington, VA, USA: American Psychiatric Association.
4 Baig MR, Navaira E, Escamilla MA, Raventos H, Walss-Bass C (2010). Clozapine treatment causes oxidation of proteins involved in energy metabolism in lymphoblastoid cells: a possible mechanism for antipsychotic-induced metabolic alterations. J Psychiatr Pract. **16**: 325–333.
5 Bang P, Eriksson U, Sara V, Wivall I-L, Hall K (1991). Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: improvement of determinations in acid ethanol extracts by the use of truncated IGF-I as radioligand. Acta Endocrinol (Copenh). **124**: 620–629.
6 Baruah S, Waziri R, Hegwood TS, Mallis LM (1991). Plasma serine in schizophrenics and controls measured by gas chromatography-mass spectrometry. Psychiatr Res. **37**: 261–270.
7 Bjerkenstedt L, Edman G, Hagenfeldt L, Sedvall G, Wiesel F-A (1985). Plasma amino acids in relation to cerebrospinal fluid monoamine metabolites in schizophrenic patients and healthy controls. Br J Psychiatry. **147**: 276–282.
8 Bloxan DL, Warren WH (1974). Error in the determination of tryptophan by the method of Denkla and Dewey. A revised procedure. Anal Biochem. **60**: 621–625.
9 Bongiovanni R, Leonard S, Jaskiw GE (2013). A simplified method to quantify dysregulated tyrosine transport in schizophrenia. Schizophr Res. **150**: 386–391.
10 Brosnan ME, Brosnan JT (2009). Hepatic glutamate metabolism: a tale of 2 hepatocytes[1–4]. Am J Clin Nutr. **90**: 857S–861S.
11 Bruneau EG, McCullumsmith RE, Haroutunian V, Davis KL, Meador-Woodruff JH (2005). Increased expression of glutaminase and glutamine synthetase mRNA in the thalamus in schizophrenia. Schizophr Res. **75**: 27–34.
12 Burbaeva GSh, Boksha IS, Turishcheva MS, Vorobyeva EA, Savushkina OK, Tereshkina EB (2003). Glutamine synthetase and glutamate dehydrogenase in the prefrontal cortex of patients with schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry. **27**: 675–680.
13 Bustillo JR, Rowland LM, Mullins P, Jung R, Chen H, Qualls C, *et al.* (2010). ^{1}H-MRS at 4 Tesla in minimally treated early schizophrenia. Mol Psychiatry. **15**: 629–636.
14 Carlsson A (1978). Antipsychotic drugs, neurotransmitters, and schizophrenia. Am J Psychiatry. **135**: 164–173.
15 Choi TB, Pardridge WM (1986). Phenylalanine transport at the human blood-brain barrier. J Biol Chem. **261**: 6536–6541.
16 Christensen HN (1990). Role of amino acid transport and countertransport in nutrition and metabolism. Phys Rev. **70**: 43–77.
17 DeFronzo RA, Ferrannini E (1992). Insulin actions in vivo: protein metabolism. In: Alberti KGMM, DeFronzo RA, Keen H, Zimmet P, editors. International textbook of diabetes mellitus. New York, USA: John Wiley & Sons Ltd. p. 467–511.
18 Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD (2012). Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. Am J Psychiatry. **169**: 1203–1210.
19 Denckla WD, Dewey HK (1967). The determination of tryptophan in plasma, liver, and urine. J Lab Clin Med. **69**: 160–169.
20 Do KQ, Lauer CJ, Schreiber W, Zollinger M, Gutteck-Amsler U, Cuénod M, *et al.* (1995). γ-Glutamylglutamine and taurine concentrations are decreased in the cerebrospinal fluid of drug-naive patients with schizophrenic disorders. J Neurochem. **65**: 2652–2662.
21 Egerton A, Brugger S, Raffin M, Barker GJ, Lythgoe DJ, McGuire PK, *et al.* (2012). Anterior cingulate glutamate levels related to clinical status following treatment in first-episode schizophrenia. Neuropsychopharmacology. **37**: 2515–2521.
22 Evins AE, Amico ET, Shih V, Goff DC (1997). Clozapine treatment increases serum glutamate and aspartate compared to conventional neuroleptics. J Neural Transm. **104**: 761–766.
23 Farde L, Wiesel F-A, Nordström A-L, Sedvall G (1989). D_1- and D_2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology. **99**: S28–S31.
24 Faustman WO, Bardgett M, Faull KF, Pfefferbaum A, Csernansky JG (1999). Cerebrospinal fluid glutamate inversely correlates with positive symptom severity in unmedicated male schizophrenic/ schizoaffective patients. Biol Psychiatry. **45**: 68–75.
25 Flyckt L, Venizelos N, Edman G, Bjerkenstedt L, Hagenfeldt L, Wiesel F-A (2001). Aberrant tyrosine transport across the cell membrane in patients with schizophrenia. Arch Gen Psychiatry. **58**: 953–958.
26 Frohlich J, Van Horn JD (2014). Reviewing the ketamine model for schizophrenia [review]. J Psychopharmacol. **28**: 287–302.
27 Fuchs SA, De Barse MMJ, Scheepers FE, Cahn W, Dorland L, de Sain-van der Velden MG, *et al.* (2008). Cerebrospinal fluid D-serine and glycine concentrations are unaltered and unaffected by olanzapine therapy in male schizophrenic patients. Eur Neuropsychopharmacol. **18**: 333–338.
28 Gattaz WF, Roberts E, Beckmann H (1986). Cerebrospinal fluid concentrations of free GABA in schizophrenia: no changes after haloperidol treatment. J Neural Transm. **66**: 69–73.
29 Gluck MR, Thomas RG, Davis KL, Haroutunian V (2002). Implications for altered glutamate and GABA metabolism in the dorsolateral prefrontal cortex of aged schizophrenic patients. Am J Psychiatry. **159**: 1165–1173.
30 Goff DC, Tsai G, Lewitt J, Amico E, Manoach D, Schoenfeld DA, *et al.* (1999). A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatry. **56**: 21–27.
31 Hagenfeldt L, Venizelos N, Bjerkenstedt L, Wiesel F-A (1987). Decreased tyrosine transport in fibroblasts from schizophrenic patients. Life Sci. **41**: 2749–2757.
32 Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindström LH, Iyo M (2005). Elevated glutamine/ glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. BMC Psychiatry. **5**: 6; doi:10.1186/1471-244X-5-6.
33 He Y, Yu Z, Giegling I, Xie L, Hartmann AM, Prehn C, *et al.* (2012). Schizophrenia shows a unique metabolomics signature in plasma. Transl Psychiatry. **2**: e149; doi:10.1038/tp.2012.76.
34 Henderson DC, Fan X, Sharma B, Copeland PM, Borba CPC, Freudenreich O, *et al.* (2009). Waist circumference is the best anthropometric predictor for insulin resistance in nondiabetic patients with schizophrenia treated with clozapine but not olanzapine. J Psychiatr Pract. **15**: 251–261.
35 Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Horowitz A, Kelly D (1996). Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. Br J Psychiatry. **169**: 610–617.
36 Hilding A, Hall K, Wivall-Helleryd I-L, Sääf M, Melin A-L, Thoren M (1999). Serum levels of insulin-like growth factor I in 152 patients with growth hormone deficiency, aged 19–82 years, in relation to those in healthy subjects. J Clin Endocrinol Metab. **84**: 2013–2019.
37 Howes OD, Bhatnagar A, Gaughran FP, Amiel SA, Murray RM, Pilowsky LS (2004). A prospective study of impairment in glucose control caused by clozapine without changes in insulin resistance. Am J Psychiatry. **161**: 361–363.
38 Jacobs S (1966). Determination of amino acids by ion exchange chromatography. Methods Biochem Anal. **14**: 177–202.
39 Kanai Y, Segawa H, Miyamoto K-i, Uchino H, Takeda E, Endou H (1998). Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J Biol Chem. **273**: 23629–23632.
40 Kilberg MS, Barber EF, Handlogten ME (1985). Characteristics and hormonal regulation of amino acid transport system A in isolated rat hepatocytes. Curr Top Cell Regul. **25**: 133–163.
41 Kimball SR, Vary TC, Jefferson LS (1994). Regulation of protein synthesis by insulin [review]. Annu Rev Physiol. **56**: 321–348.
42 Koolman J, Roehm K-H (2013). Color Atlas of Biochemistry, 3rd edition. Stuttgart, Germany: Georg Thieme Verlag KG.

43 Labhart A (1986). Classification of diabetes mellitus. In: Thorn GW, Assal JP, editors. Clinical Endocrinology: Theory and Practice. Berlin, Germany: Springer-Verlag. p. 775–777.
44 Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R (1999). Increased dopamine transmission in schizophrenia: relationship to illness phases. Biol Psychiatry. **46**: 56–72.
45 Lee M, Jayathilake K, Dai J, Meltzer HY (2011). Decreased plasma tryptophan and tryptophan/ large neutral amino acid ratio in patients with neuroleptic-resistant schizophrenia: Relationship to plasma cortisol concentration. Psychiatry Res. **185**: 328–333.
46 Lewitt MS, Hilding A, Brismar K, Efendic S, Östenson C-G, Hall K (2010). IGF-binding protein 1 and abdominal obesity in the development of type 2 diabetes in women. Eur J Endocrinol. **163**: 233–242.
47 Lewitt MS, Hilding A, Östenson C-G, Efendic S, Brismar K, Hall K (2008). Insulin-like growth factor-binding protein-1 in the prediction and development of type 2 diabetes in middle-aged Swedish men. Diabetologia. **51**: 1135–1145.
48 Malhotra N, Grover S, Chakrabarti S, Kulhara P (2013). Metabolic syndrome in schizophrenia [review]. Indian J Psychol Med. **35**: 227–240.
49 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. **28**: 412–419.
50 Melkersson KI, Dahl M-L (2003). Relationship between levels of insulin or triglycerides and serum concentrations of the atypical antipsychotics clozapine and olanzapine in patients on treatment with therapeutic doses. Psychopharmacology. **170**: 157–166.
51 Melkersson KI, Dahl M-L, Hulting A-L (2004). Guidelines for prevention and treatment of adverse effects of antipsychotic drugs on glucose-insulin homeostasis and lipid metabolism [review]. Psychopharmacology. **175**: 1–6.
52 Melkersson KI, Hulting A-L, Brismar KE (1999). Different influences of classical antipsychotics and clozapine on glucose-insulin homeostasis in patients with schizophrenia or related psychoses. J Clin Psychiatry. **60**: 783–791.
53 Melkersson KI, Hulting A-L, Rane AJ (2001). Dose requirement and prolactin elevation of antipsychotics in male and female patients with schizophrenia or related psychoses. Br J Clin Pharmacol. **51**: 317–324.
54 Olney JW, Farber NB (1995). Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry. **52**: 998–1007.
55 Oxender DL, Christensen HN (1963). Evidence for two types of mediation of neutral amino-acid transport in Ehrlich cells. Nature. **97**: 765–767.
56 Papouin T, Ladépêche L, Ruel J, Sacchi S, Labasque M, Hanini M, *et al.* (2012). Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell. **150**: 633–646.
57 Paredes RM, Quinones M, Marballi K, Gao X, Valdez C, Ahuja SS, *et al.* (2014). Metabolomic profiling of schizophrenia patients at risk for metabolic syndrome. Int J Neuropsychopharmacol. **17**: 1139–1148.
58 Perry TL, Hansen S (1984). Interconversion of serine and glycine is normal in psychotic patients. Psychiatr Res. **15**: 109–113.
59 Pineda M, Fernandez E, Torrents D, Estevez R, Lopez C, Camps M, *et al.* (1999). Identification of a membrane protein, LAT-2, that co-expresses with 4F2 heavy chain, an L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. J Biol Chem. **274**: 19738–19744.
60 Potkin SG, Cannon-Spoor HE, DeLisi LE, Neckers LM, Wyatt RJ (1983). Plasma phenylalanine, tyrosine, and tryptophan in schizophrenia. Arch Gen Psychiatry. **40**: 749–752.
61 Potkin SG, Jin Y, Bunney BG, Costa J, Gulasekaram B (1999). Effect of clozapine and adjunctive high-dose glycine in treatment-resistant schizophrenia. Am J Psychiatry. **156**: 145–147.
62 Povoa G, Roovete A, Hall K (1984). Cross-reaction of serum somatomedin-binding protein in a radioimmunoassay developed for somatomedin-binding protein isolated from human amniotic fluid. Acta Endocrinol (Copenh). **107**: 563–570.
63 Ramchand CN, Peet M, Clark AE, Gliddon AE, Hemmings GP (1996). Decreased tyrosine transport in fibroblasts from schizophrenics: implications for membrane pathology. Prostaglandins Leukot Essent Fatty Acids. **55**: 59–64.
64 Rettenbacher MA, Hummer M, Hofer A, Baumgartner S, Ebenbichler C, Edlinger M, *et al.* (2007). Alterations of glucose metabolism during treatment with clozapine or amisulpride: results from a prospective 16-week study. J Psychopharmacol. **21**: 400–404.
65 Roberts RC, Roche JK, Conley RR, Lahti AC (2009). Dopaminergic synapses in the caudate of subjects with schizophrenia: relationship to treatment response. Synapse. **63**: 520–530.
66 Scheepers FE, Gispen-de Wied CC, Westenberg HGM, Kahn RS (2002). Effect of olanzapine on glutamate levels in cerebrospinal fluid of patients with schizophrenia [letter]. J Clin Psychopharmacol. **22**: 333–334.
67 Scott CR (2006). The genetic tyrosinemias [review]. Am J Med Genet. **142C**: 121–126.
68 Seeman MV, Seeman P (2014). Is schizophrenia a dopamine supersensitivity psychotic reaction? Prog Neuropsychopharmacol Biol Psychiatry. **48**: 155–160.
69 Struys EA (2006). D-2-Hydroxyglutaric aciduria: unravelling the biochemical pathway and the genetic defect. J Inherit Metab Dis. **29**: 21–29.
70 Théberge J, Bartha R, Drost DJ, Menon RS, Malla A, Takhar J, *et al.* (2002). Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. Am J Psychiatry. **159**: 1944–1946.
71 Théberge J, Williamson KE, Aoyama N, Drost DJ, Manchanda R, Malla AK, *et al.* (2007). Longitudinal grey-matter and glutaminergic losses in first-episode schizophrenia. Br J Psychiatry. **191**: 325–334.
72 Tortorella A, Monteleone P, Fabrazzo M, Viggiano A, De Luca L, Maj M (2001). Plasma concentrations of amino acids in chronic schizophrenics treated with clozapine. Neuropsychobiology. **44**: 167–171.
73 Tsai G, van Kammen DP, Chen S, Kelley ME, Grier A, Coyle JT (1998). Glutaminergic neurotransmission involves structural and clinical deficits of schizophrenia. Biol Psychiatry. **44**: 667–674.
74 Tsai GE, Yang P, Chung L-C, Tsai I-C, Tsai C-W, Coyle JT (1999). D-serine added to clozapine for the treatment of schizophrenia. Am J Psychiatry. **156**: 1822–1825.
75 Walss-Bass C, Weintraub ST, Hatch J, Mintz J, Chaudhuri AR (2008). Clozapine causes oxidation of proteins involved in energy metabolism: a possible mechanism for antipsychotic-induced metabolic alterations. Int J Neuropsychopharmacol. **11**: 1097–1104.
76 Varoqui H, Zhu H, Yao D, Ming H, Erickson JD (2000). Cloning and functional identification of a neuronal glutamine transporter. J Biol Chem. **275**: 4049–4054.
77 Wiesel F-A, Andersson JLR, Westerberg G, Wieselgren I-M, Bjerkenstedt L, Hagenfeldt L, *et al.* (1999). Tyrosine transport is regulated differently in patients with schizophrenia. Schizophr Res. **40**: 37–42.
78 Wiesel F-A, Blomqvist G, Halldin C, Sjögren I, Bjerkenstedt L, Venizelos N, *et al.* (1991). The transport of tyrosine into the human brain as determined with L-[1-^{11}C] tyrosine and PET. J Nucl Med. **32**: 2043–2049.
79 Wiesel F-A, Venizelos N, Bjerkenstedt L, Hagenfeldt L (1994). Tyrosine transport in schizophrenia. Schizophr Res. **13**: 255–258.
80 Vumma R, Wiesel F-A, Flyckt L, Bjerkenstedt L, Venizelos N (2008). Functional characterization of tyrosine transport in fibroblast cells from healthy controls. Neurosci Lett. **434**: 56–60.
81 Wurtman RJ, Hefti F, Melamed E (1980). Precursor control of neurotransmitter synthesis. Pharmacol Rev. **32**: 315–335.
82 Xuan J, Pan G, Qui Y, Yang L, Su M, Liu Y, *et al.* (2011). Metabolomic profiling to identify potential serum biomarkers for schizophrenia and risperidone action. J Proteom Res. **10**: 5433–5443.
83 Yanagida O, Kanai Y, Chairoungdua A, Kim DK, Segawa H, Nii T, *et al.* (2001). Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. Biochim Biophys Acta. **1514**: 291–302.
84 Yaryura-Tobias JA, Chang A, Neziroglu F (1978). A study of the relationships of serum glucose, insulin, free fatty acids, and free and total tryptophan to mental illness. Biol Psychiatry. **13**: 243–254.