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ARTICLE

# Metabolomic alteration induced by psychotropic drugs: Short-term metabolite profile as a predictor of weight gain evolution

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**Abstract**

Psychotropic drugs can induce strong metabolic adverse effects, potentially increasing morbidity and/or mortality of patients. Metabolomic profiling, by studying the levels of numerous metabolic intermediates and products in the blood, allows a more detailed examination of metabolism dysfunctions. We aimed to identify blood metabolomic markers associated with weight gain in psychiatric patients. Sixty-two patients starting a treatment known to induce weight gain were recruited. Two hundred and six selected metabolites implicated in various pathways were analyzed in plasma, at baseline and after 1 month of treatment. Additionally, 15 metabolites of the kynurenine pathway were quantified. This latter analysis was repeated in a confirmatory cohort of 24 patients. Among the 206 metabolites, a plasma metabolomic fingerprint after 1 month of treatment embedded 19 compounds from different chemical classes (amino acids, acylcarnitines, carboxylic acids, catecholamines, nucleosides, pyridine, and tetrapyrrole) potentially involved in metabolic disruption and inflammation processes. The predictive potential of such early metabolite changes on 3 months of weight evolution was then explored using a linear mixed-effects model. Of these 19 metabolites, short-term modifications of kynurenine, hexanoylcarnitine, and biliverdin, as well as kynurenine/tryptophan ratio at 1 month, were associated with 3 months weight evolution. Alterations of the kynurenine pathway were confirmed by quantification, in both exploratory and confirmatory cohorts. Our metabolomic study suggests a specific metabolic dysregulation after 1 month of treatment with psychotropic drugs known to induce weight gain. The identified metabolomic signature could contribute in the future to the prediction of weight gain in patients treated with psychotropic drugs.

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**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**
Psychotropic drugs can induce weight gain, potentially increasing morbidity and/or mortality of patients.

**WHAT QUESTION DID THIS STUDY ADDRESS?**
Could the use of metabolomics permit the identification of early blood markers associated with weight gain in treated psychiatric patients?

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**
It identified a metabolomic signature related to psychotropic drug exposure. Our results suggest an overall metabolic dysregulation after 1 month of treatment, and a specific dysregulation that is associated with weight gain worsening at 3 months. Understanding the biochemical significance of those dysregulations should provide further mechanistic comprehensions of pathways involved in metabolic effects of psychotropic drugs and possibly also in therapeutic response to treatments.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**
Metabolomic profiling could be used in a combinatorial model with clinical, exposure, and genetic markers, to improve the early prediction of weight gain during psychotropic treatment. This prediction could be useful to offer personalized treatment to patients and, consequently, improved medical care and patient quality of life.

## INTRODUCTION

Psychotropic drugs are widely prescribed in psychiatric patients with proven efficacy. However, most atypical as well as some typical antipsychotics, mood stabilizers, and antidepressants can induce important metabolic side effects, including weight gain (WG).[1] Psychotropic drug-induced WG can thus lead to several metabolic complications, including dyslipidemia, diabetes, and/or hypertension, partly responsible for the increase of cardiovascular diseases and reduction of lifespan by 10–15 years in this population.[2,3] In addition to psychiatric illness itself, evidence suggests that additional factors, including female gender, low baseline body mass index (BMI), young age, first episode of psychiatric illness, and/or drug naïve patients, are associated with an increased risk of drug-induced WG.[4]

Underlying mechanisms of psychotropic drug-induced WG are only partially understood. The involvement of many neurotransmitters and neuropeptides, such as neuropeptide-Y (NPY), orexin, or melanin-concentrating hormone (MCH),[5] of hormones, such as leptin, adiponectin, insulin, and ghrelin,[6] as well as of genetic factors,[7] has been reported. At the cellular level, alterations in the mitochondrial structure and functions, such as alterations in electron transport, impairment of oxidative phosphorylation, and tricarboxylic acid cycle, leading to dysregulation of energy metabolism, have also been incriminated.[8] There is thus a growing interest in identifying clinical and biological alterations associated with psychotropic medication use, which could allow for better understanding of the multiple mechanisms involved in drug-induced WG and/or worsening of other metabolic parameters. In addition, the detection of early alterations of such biomarkers would allow one to predict metabolic outcomes and to propose individualization of treatment. In this context, a WG higher than 5% after 1 month of treatment with weight-inducing psychotropic drugs was demonstrated to be a clinical sign to consider patients as being at higher risk of significant WG during long-term treatment.[9] Likewise, the increase of lipid levels by more than 5% after 1 month of treatment is the best predictor for dyslipidemia at a later stage.[10]

Metabolomics is a powerful phenotyping approach assimilating alterations in all small molecules and metabolites produced by the body, influenced by physiological, pathological, or environmental states, including drug exposure.[11] As the metabolome is downstream of the cellular process, it is the closest link to its phenotype, providing dynamic and sensitive information on its changes. Its exploration could permit identifying metabolic fingerprints used as biomarkers, thus predicting a particular phenotype. When possible, pathway exploration of

metabolites involved in this fingerprint could provide new insights into mechanisms that generate this phenotype.[12,13] Recent advances in mass spectrometry technology enable high sensitivity and broad coverage of the metabolome.[14] Moreover, high throughput analysis permits rapid performance of large-scale studies of hundreds of metabolites, showing an undoubted advantage compared to analysis targeting only one metabolite or one pathway.

A series of investigations has demonstrated the feasibility and benefits of metabolomic analyses in psychiatric populations for responding to several specific issues. Metabolomic fingerprints have been described for differentiating several pathologies in the psychiatric population, providing potential biomarkers for facilitating diagnosis, exploring new physiopathology and treatment hypotheses.[15,16] Studies focusing on variations of the metabolome after pharmacotherapy shed light on various metabolic dysregulations.[17] Notably, disturbances in amino acid metabolism, antioxidant defense system, fatty acid biosynthesis, and/or phospholipid metabolism were observed, leading to global energy metabolism dysregulations after psychotropic drug treatment.[18,19] Interestingly, metabolomic profiles after treatment were shown to differ depending on psychotropic drugs, inducing different metabolic side effects during their administration.[20] The predictive potential of a metabolic fingerprint profile before the instauration of the treatment for early personalized intervention is more sparsely studied. Specifically, regarding the WG issue, a link between WG and increased baseline levels of triacylglycerols with low carbon number and double-bound count was observed via lipidomic analysis.[21] More generally, the list of reproducible findings is limited, reflecting the complexity of mechanisms involved in WG during the use of psychotropic medications. We hypothesized that important and early (i.e., after 1 month) modifications in metabolite profile might occur after initiation of a WG-inducing psychotropic treatment, and that such modifications could be predictive of the extent of weight change in the longer term. For this purpose, we performed targeted metabolomic analysis on plasma samples from patients starting WG-inducing psychotropic drugs in order to bring out the modifications in metabolite profiles between pre- (i.e., before) and post-treatment (i.e., after 1 month). We then aimed at identifying a predictive fingerprint for WG that could be a relevant biomarker used in personalized medicine. With the identified metabolomic signature, we then investigated the predictive value of baseline metabolite levels and of metabolite changes after 1 month of treatment on weight evolution after 1 month and over 3 months of treatment, respectively. Of note, due to the targeted nature of our metabolomic analysis, mechanistic analysis via metabolic pathway analysis was not performed.

# METHODS

## Study subjects and samples

An exploratory cohort of 62 patients was selected from an ongoing longitudinal observational study (PsyMetab) launched in 2007 in the Department of Psychiatry of the Lausanne University Hospital.[22] A second cohort of 24 patients, also from the PsyMetab study, was secondarily included as a confirmatory group. Written informed consent was given by all subjects or their legal representatives. The study was approved by the ethics committee of the State of Vaud (CER-VD).

Patients started a treatment with one or several WG-inducing psychotropic drugs. The inclusion period of patients in the first and second cohorts was between May 29, 2013, and June 5, 2016, and between August 26, 2014, and June 23, 2017, respectively. The prescribed doses of all psychotropic drugs were within the clinically recommended range and monitored through therapeutic drug monitoring, enabling control of compliance and pharmacokinetic issues, including rapid metabolism and/or drug interactions. Baseline and 1-month clinical and biological data were recorded during a medical examination based on guidelines.[23] Only hospitalized patients were included in order to ascertain fasting conditions and rapid processing of samples after blood drawing (centrifugation at 4°C, aliquoting and storage at −80°C until analysis). Patients in the first and second cohorts were selected as having either less than 5% WG ($n = 44$ and $n = 16$, respectively) or equal to or higher than 5% WG ($n = 18$ and $n = 8$, respectively) after 1 month of treatment. Additional weight measures were recorded, depending on the hospital-based follow-up of each patient, during the first 100 days following treatment initiation. Scarcity of data after this period did not permit analysis of longer follow-up.

## Targeted metabolomic analysis

The 124 plasma samples of the exploratory cohort were prepared with a deproteinization step carried out under cold conditions. The 100 µl was deproteinized with 300 µl of a solution containing methanol and ethanol (1:1, v/v), vortexed and centrifuged at 14,000 g at +8°C for 15 min. Supernatants were collected and dried with speedvac and then reconstituted in 100 µl of a high aqueous solvent (water/10% methanol) before injecting 5 µl for analysis.

Based on published protocol,[24] a targeted metabolomic analysis was developed, as previously described,[25] allowing the detection of 206 metabolites (Table S1). The detailed description of the experimental procedures can be found in the Supplementary Material. Data processing was performed through MultiQuant software (version 2.0, Sciex). Relative intensities were obtained by comparing peak intensities of different studied conditions and were analyzed using MetaboAnalyst 3.0.[26] In particular, results were normalized by sum, log transformation, and auto-scaling to obtain a normally distributed population. First, a metabolomic signature, which differentiates between patients in pre- and post-treatment, was searched using a Student's paired *t*-test with the same software. A significance threshold was set at a *p* value of 0.05 after adjustment for the false discovery rate (FDR)[27] to correct for multiple statistical testing. Significant metabolites were considered to be a metabolomic signature resulting from a 1-month psychotropic drug treatment. All consecutive statistical analyses were performed using R language and environment for statistical computing.[28] A significance threshold was set at a *p* value of 0.05 after adjustment by sex, age, and FDR. Thus, second, considering the identified metabolomic signature, the link between baseline metabolite levels and 1-month weight change was assessed by linear regression. Third, to explore the predictive effect of metabolite changes during short-term psychotropic drug treatment (1 month) on a 3 month-weight evolution, we performed a linear mixed-effect model.[29] Patients were separated into two groups according to the median value of each metabolite change after 1 month of treatment. WG observations between baseline and 3 months of treatment were used to fit the models (Table S2). Those models were adjusted for repeated measurements by including a random effect for each individual. Significant metabolites were considered to be predictive of intermediate-term weight evolution.

## Exploration of the kynurenine pathway

A validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method was applied to the 172 plasma samples of the exploratory and confirmatory cohorts, as described previously.[30] This method allows the identification and quantification of tryptophan and 14 metabolites from the kynurenine pathway (KP) and is described in the Supplementary Material. Data processing was performed through MassLynx software (version 4.2, Waters). Concentration results were analyzed using MetaboAnalyst 3.0[26] and R.[28] A significance threshold was set at a *p* value of 0.05. Adjustment by FDR was applied for the exploratory cohort, but not for the confirmation cohort. The same methodology as described previously was used first to identify metabolites from the KP contributing to the differentiation of patients between pre- and post-treatment, second, the link between baseline identified metabolite levels and 1-month weight evolution and, third, the relation between WG over 3 months and 1-month variations of those metabolites.

# RESULTS

## Subject characteristics

The mean age (±SD) of the discovery and confirmatory cohorts was 37.5 (±12.5) years and 52.1 (±22.9) years, respectively. Demographic and clinical data are presented in Table 1. In addition to the expected increases in weight and BMI, total cholesterol, including low-density lipoprotein cholesterol, was significantly increased after 1 month of treatment in the discovery cohort, which is consistent with metabolic side effects frequently observed under psychotropic drug treatment. Surprisingly, systolic and diastolic blood pressure decreased after 1 month of treatment, potentially explained by higher stress at admission. Ten patients among the exploratory cohort were drug-naïve patients but did not show any significant difference in their baseline metabolite expression (data not shown). Of note, olanzapine was the most frequently prescribed psychotropic drug.

## Targeted metabolomic analysis

Nineteen of 206 metabolites were retained after *t*-test analyses, highlighting a metabolomic signature that differentiates patients between pre- and post-treatment in the exploratory cohort (Table 2). Multivariate partial least squares discriminant analysis (PLS-DA) was also performed using Metaboanalyst 5.0, and confirmed the present results. Thus, 95% of metabolites, which are significant in *t*-test analysis, corrected by FDR, were classified at the top of the highest Variable Importance in the Projection score (VIP >1.5; data not shown). Significant metabolites belong to various biochemical classes: amino acids ($n = 5$), acylcarnitines ($n = 6$), carboxylic acids ($n = 4$), catecholamines ($n = 1$), nucleosides ($n = 1$), pyridine ($n = 1$), and tetrapyrrole ($n = 1$). Seven metabolites, L-alanine, kynurenine, 6-hydroxydopamine, *N*-amidino-L-aspartate, pyridoxamine, propionylcarnitine, and creatine, were increased after 1 month of treatment. The kynurenine/tryptophan ratio has previously been associated with the enzymatic activity of indoleamine-2,3-dioxygenase, a tryptophan-degrading enzyme with enhanced activity under inflammation status. In this context, the kynurenine/tryptophan ratio

**TABLE 1** Demographic and clinical characteristics of the patients at baseline and after 1 month of treatment

| | Discovery cohort | | | Confirmatory cohort | | |
|---|---|---|---|---|---|---|
| **Demographic** | **Baseline** | **T + 1 month** | ***p* value** | **Baseline** | **T + 1 month** | ***p* value** |
| Age mean ± SD (years) | 37.5 ± 12.5 ($n = 62$) | — | — | 52.1 ± 22.9 ($n = 24$) | — | — |
| Gender, % of males | 50.0 ($n = 62$) | — | — | 45.8 ($n = 24$) | — | — |
| Currently smoking, % | 60.7 ($n = 62$) | 65.0 ($n = 60$) | — | — | — | — |
| Weight (kg), mean ± SD | 73.3 ± 12.5 ($n = 62$) | 76.0 ± 12.8 ($n = 62$) | $6.4E^{-9}$* ($n = 62$) | 70.3 ± 18.3 ($n = 24$) | 73.5 ± 19.2 ($n = 24$) | 0.0001* ($n = 24$) |
| Body Mass Index mean ± SD ($kg/m^2$) | 25.0 ± 4.6 ($n = 61$) | 26.0 ± 4.7 ($n = 61$) | $4.3E^{-9}$* ($n = 61$) | 24.6 ± 6.0 ($n = 24$) | 25.7 ± 6.2 ($n = 24$) | 0.0001* ($n = 24$) |
| Systolic blood pressure mean ± SD (mmHg) | 125.7 ± 16.4 ($n = 58$) | 120.0 ± 14.6 ($n = 21$) | 0.03* ($n = 21$) | 125.2 ± 17.6 ($n = 20$) | 120.0 ± 17.3 ($n = 5$) | 0.84 ($n = 5$) |
| Diastolic blood pressure mean ± SD (mmHg) | 80.9 ± 9.8 ($n = 58$) | 73.3 ± 8.1 ($n = 21$) | 0.0003* ($n = 21$) | 81.5 ± 14.5 ($n = 20$) | 71.6 ± 12.7 ($n = 5$) | 0.55 ($n = 5$) |
| Glucose mean ± SD (mmol/L) | 5.2 ± 0.8 ($n = 55$) | 5.3 ± 1.0 ($n = 20$) | 0.52 ($n = 20$) | 5.2 ± 0.9 ($n = 15$) | 6.0 ± 0.9 ($n = 7$) | 0.12 ($n = 4$) |
| Total cholesterol mean ± SD (mmol/L) | 4.5 ± 1.0 ($n = 56$) | 5.1 ± 1.1 ($n = 51$) | 0.0008* ($n = 47$) | 4.7 ± 1.2 ($n = 17$) | 4.8 ± 1.0 ($n = 19$) | 0.58 ($n = 14$) |
| High density lipoprotein cholesterol mean ± SD (mmol/L) | 1.3 ± 0.3 ($n = 54$) | 1.3 ± 0.4 ($n = 52$) | 0.73 ($n = 47$) | 1.5 ± 0.5 ($n = 17$) | 1.3 ± 0.4 ($n = 19$) | 0.46 ($n = 14$) |
| Low density lipoprotein cholesterol mean ± SD (mmol/L) | 3.0 ± 0.9 ($n = 54$) | 3.5 ± 1.0 ($n = 51$) | 0.002* ($n = 45$) | 3.0 ± 1.1 ($n = 17$) | 3.2 ± 1.1 ($n = 19$) | 0.19 ($n = 14$) |
| Triglycerides mean ± SD (mmol/L) | 1.3 ± 0.7 ($n = 54$) | 1.5 ± 0.9 ($n = 53$) | 0.33 ($n = 47$) | 1.1 ± 0.6 ($n = 17$) | 1.1 ± 0.5 ($n = 19$) | 0.44 ($n = 14$) |
| Medication (%) | | | | | | |
| Olanzapine | 20.8 | — | — | 20.8 | — | — |
| Risperidone | 16.1 | — | — | 4.2 | — | — |
| Amisulpride | 11.2 | — | — | 0 | — | — |
| Quetiapine | 11.2 | — | — | 8.3 | — | — |
| Aripiprazole | 9.6 | — | — | 4.2 | — | — |
| Lithium | 8.1 | — | — | 8.3 | — | — |
| Haloperidol | 6.5 | — | — | 20.8 | — | — |
| Zuclopenthixol | 6.5 | — | — | 8.3 | — | — |
| Clozapine | 3.3 | — | — | 8.3 | — | — |
| Valproate | 3.3 | — | — | 8.3 | — | — |
| Mirtazapine | 1.7 | — | — | 4.2 | — | — |
| Paliperidone | 1.7 | — | — | 4.2 | — | — |
| More than one psychotropic drug from the above list | 64.5 | — | | 75.0 | — | |

*Note:* Values are indicated as mean ± SD. Significant difference at paired *t*-test are indicated with a star (*$p < 0.05$).

was also evaluated and showed a significant increase after 1 month of treatment. In contrast, the 12 remaining metabolites were decreased after 1 month of treatment. Neither metabolite nor the kynurenine/tryptophan ratio were significantly associated with WG during the first month of treatment (data not shown) when assessed by a linear regression analysis on the baseline values of the above-mentioned metabolites. Using linear mixed-effect models, the 1-month variations of four metabolites were associated with WG over 3 months (Figure 1). Thus, patients with a variation in kynurenine ($p = 0.046$) or kynurenine/tryptophan ratio ($p = 0.046$), and in biliverdin ($p = 0.046$) or hexanoylcarnitine ($p = 0.046$), after 1 month of treatment, higher and lower, respectively, than the median changes had significantly higher WG during the first 3 months of treatment.

## Exploration of the kynurenine pathway

Metabolites of the KP have been extensively studied for its implications in psychiatric disorders as well as in metabolic dysregulations, including WG. Considering the importance of those two topics for our study, and considering the presence of kynurenine in the metabolic signature, a quantitative analysis of metabolites of the KP was performed in the exploratory cohort with a validated orthogonal method. Kynurenine ($p = 0.0005$), 5-hydroxy-tryptophan ($p = 0.02$), and the kynurenine/tryptophan ratio ($p = 0.02$) were found to be significantly increased after 1 month of treatment. Linear regression analysis did not show any association between baseline values of the aforementioned markers and WG over 1 month (data not shown). However, among them, the variation of kynurenine/tryptophan ratio over 1 month was confirmed to be associated with WG over 3 months ($p = 0.04$; Figure 2).

The same analysis achieved in the confirmatory cohort confirmed that the kynurenine level ($p = 0.004$) and the kynurenine/tryptophan ratio ($p = 0.04$) were higher after 1 month of treatment, as well as the 3-hydroxy-anthranilic acid level ($p = 0.0007$). In agreement with results from the exploratory cohort, no associations were found between baseline values of the KP metabolites and WG over 1 month, confirming no predictive value of baseline metabolites on early WG. In contrast, exploration of the relationship between 1-month variation of metabolites and

**TABLE 2** Analytes whose levels were significantly modified after one month of psychotropic drug use, and their feature classes

| Analytes | Ionization mode | HMDB ID | *t*.stat | $p_{corrected}$ | Feature class |
|---|---|---|---|---|---|
| 3-hydroxy-3-methylglutarate | Negative | HMDB00355 | 3.238 | 0.04 | Carboxylic acids |
| 6-hydroxydopamine | Positive | HMDB01537 | −3.487 | 0.01 | Catecholamines |
| Biliverdin | Positive | HMDB01008 | 3.953 | 0.004 | Tetrapyrroles |
| Creatine | Positive | HMDB00064 | −5.095 | 0.0005 | Amino acids |
| Decanoylcarnitine | Positive | HMDB00651 | 4.767 | 0.0009 | Acylcarnitine |
| Deoxyuridine | Positive | HMDB00012 | 3.615 | 0.01 | Pyrimidine nucleosides |
| D-galacturonic acid | Negative | HMDB02545 | 4.450 | 0.0009 | Carboxylic acids |
| Hexanoylcarnitine | Positive | HMDB00705 | 4.119 | 0.003 | Acylcarnitine |
| Hydroxypyruvate | Negative | HMDB01352 | 4.744 | 0.0005 | Carboxylic acids |
| Kynurenine | Positive | HMDB00684 | −4.232 | 0.002 | Amino acids |
| Kynurenine/Tryptophan ratio | Positive | — | −4.058 | 0.003 | Ratio of amino acids |
| L-alanine | Positive | HMDB00161 | −3.192 | 0.03 | Amino acids |
| Lauroylcarnitine | Positive | HMDB02250 | 4.426 | 0.002 | Acylcarnitine |
| Malonate | Negative | HMDB00691 | 5.604 | < 0.0001 | Carboxylic acids |
| *N*-Amidino-L-Aspartate | Positive | HMDB03157 | −3.230 | 0.03 | Amino acids |
| Octanoylcarnitine | Positive | HMDB00791 | 4.666 | 0.0009 | Acylcarnitine |
| Oleylcarnitine | Positive | HMDB05065 | 4.047 | 0.003 | Acylcarnitine |
| Propionylcarnitine | Positive | HMDB00824 | −3.075 | 0.03 | Acylcarnitine |
| Pyridoxamine | Positive | HMDB01431 | −3.125 | 0.03 | Pyridines |
| Thyroxine | Positive | HMDB00248 | 3.083 | 0.03 | Amino acids |

*Note:* Paired *t*-test analysis followed by FDR correction was performed between pre- and post-treatment groups. Metabolites that were not significantly different between groups are not shown.

Abbreviations: FDR, false discovery rate; HMDB, Human Metabolome DataBase.

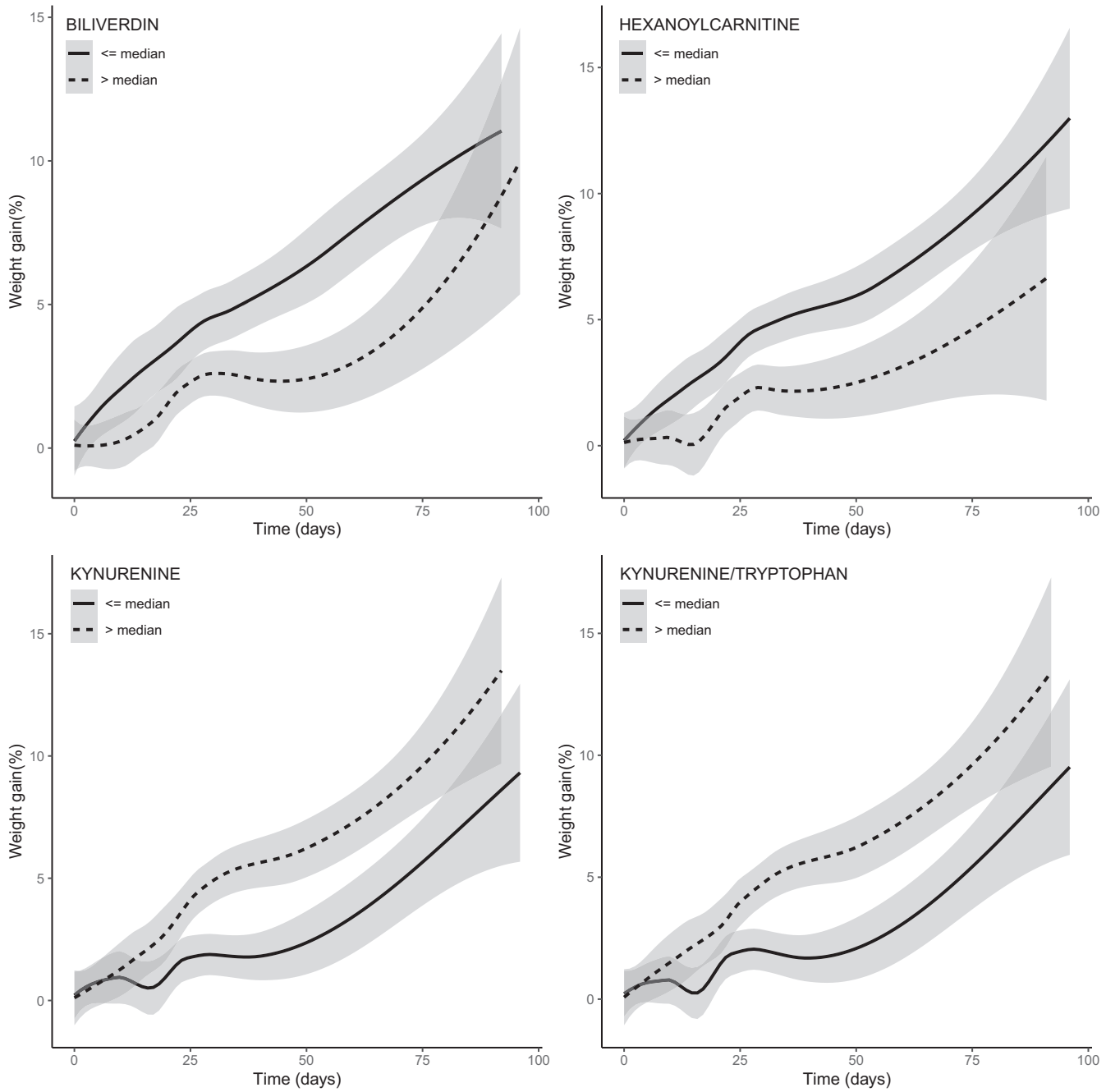


**FIGURE 1** Linear mixed-effect prediction of weight gain over 3 months performed with metabolomic results, stratified by biliverdin, hexanoylcarnitine, kynurenine, or kynurenine/tryptophan ratio. Plots represent weight changes in patients having more than (dashed lines) or up to (continuous lines) the median value of each metabolite or ratio changes after one month of treatment. The 95% confidence interval for each curve is represented by the shaded area

WG over 3 months uncovered potential trends in significance for 3-hydroxy-anthranilic acid ($p = 0.06$) and the kynurenine/tryptophan ratio ($p = 0.07$).

## DISCUSSION

Considering psychotropic-induced WG and the effects of WG on lifespan and quality of life, there is a need to identify markers that would help to predict substantial weight increase during treatment. To our knowledge, the present study is the first to achieve a comprehensive metabolic profiling of patients starting treatment with WG-inducing psychotropic drugs. With each patient acting as his or her own control, a robust metabolomic fingerprint, composed of 19 metabolites belonging to different chemical classes, was shown to be linked to 1 month of treatment. They are involved in different metabolic pathways, including energy

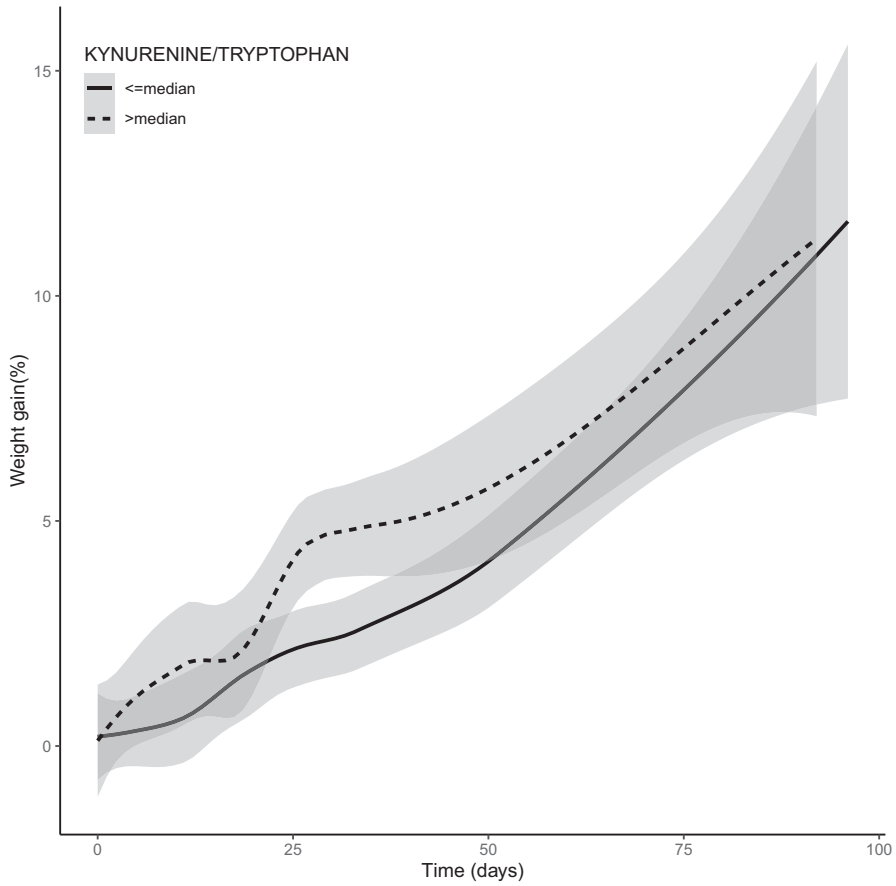


**FIGURE 2** Linear mixed-effect prediction of weight gain over 3 months performed with kynurenine pathway results, stratified by the kynurenine/tryptophan ratio. Plots represent weight changes in patients having more than (dashed line) or up to (continuous line) the median value of ratio changes after 1 month of treatment. The 95% confidence interval for each curve is represented by the shaded area

metabolism, oxidative stress, beta-oxidation, mitochondrial metabolism, amino acid metabolism, carbonyl stress, and pyrimidine metabolism, possibly reflecting new insights into metabolic changes after 1 month of psychotropic treatment.

The link between identified metabolomic fingerprint and WG under treatment was studied. An ideal biomarker should be identified before the instauration of treatment. However, baseline data from the 19 identified metabolites failed to predict weight change after 1 month. Nevertheless, we assessed whether the level changes observed during short-term treatment (1 month) could be predictors of intermediate-term (3 months) weight evolution. Interestingly, increased kynurenine levels and kynurenine/tryptophan ratios, along with decreased biliverdin and hexanoylcarnitine levels at 1 month were significantly associated with higher WG over 3 months of treatment. These findings suggest that individual metabolic responses to psychotropic drugs might be contained in early changes of the metabolome.

Tryptophan is an essential amino acid, a precursor of serotonin produced through the serotonin pathway, or of other bioactive molecules through the indolic pathway, also called KP. Its catabolism begins with its conversion into kynurenine, catalyzed by tryptophan-2,3-dioxygenase and indoleamine-2,3-dioxygenase (IDO) enzymes, the latter being inducible by a number of cytokines linked to inflammation, and other bioactive molecules.[31] Kynurenine is the starting point of the KP, which leads to nicotinamide adenine dinucleotide synthesis, a cellular energy source (Figure 3). KP metabolites have been extensively studied for their role in different physiological processes, including immunomodulation, metabolism, or neurophysiology.[32] Regarding the latter, kynurenic acid and quinolinic acid are both neuroactive. However, only tryptophan, kynurenine, and 3-hydroxykunurenine are able to cross the brain blood barrier, supporting a strong link between peripheral and central tryptophan metabolism. Dysregulation of the KP, with secondary central dysfunctions, is hypothesized to be a pathogenic mechanism of psychiatric disorders.[33] In particular, IDO expression appears to be affected by the dysregulation of pro-inflammatory cytokines associated with diseases.[34] Our metabolomic results, confirmed by a specific targeted analysis, strengthen the hypothesis of a modulation of this pathway during psychotropic drug use, which has already been described in longer term treatment follow-up.[35,36] Moreover, we underlined a supplementary variation in kynurenine/tryptophan ratio, which reflects increased enzymatic activity of IDO, possibly upregulated by inflammation state during treatment. Our results also highlighted a link between short-term variation in the KP and intermediate-term weight evolution (3 months). A similar trend was observed in our confirmation cohort, a lack of statistical significance being possibly explained by the small sample size of this

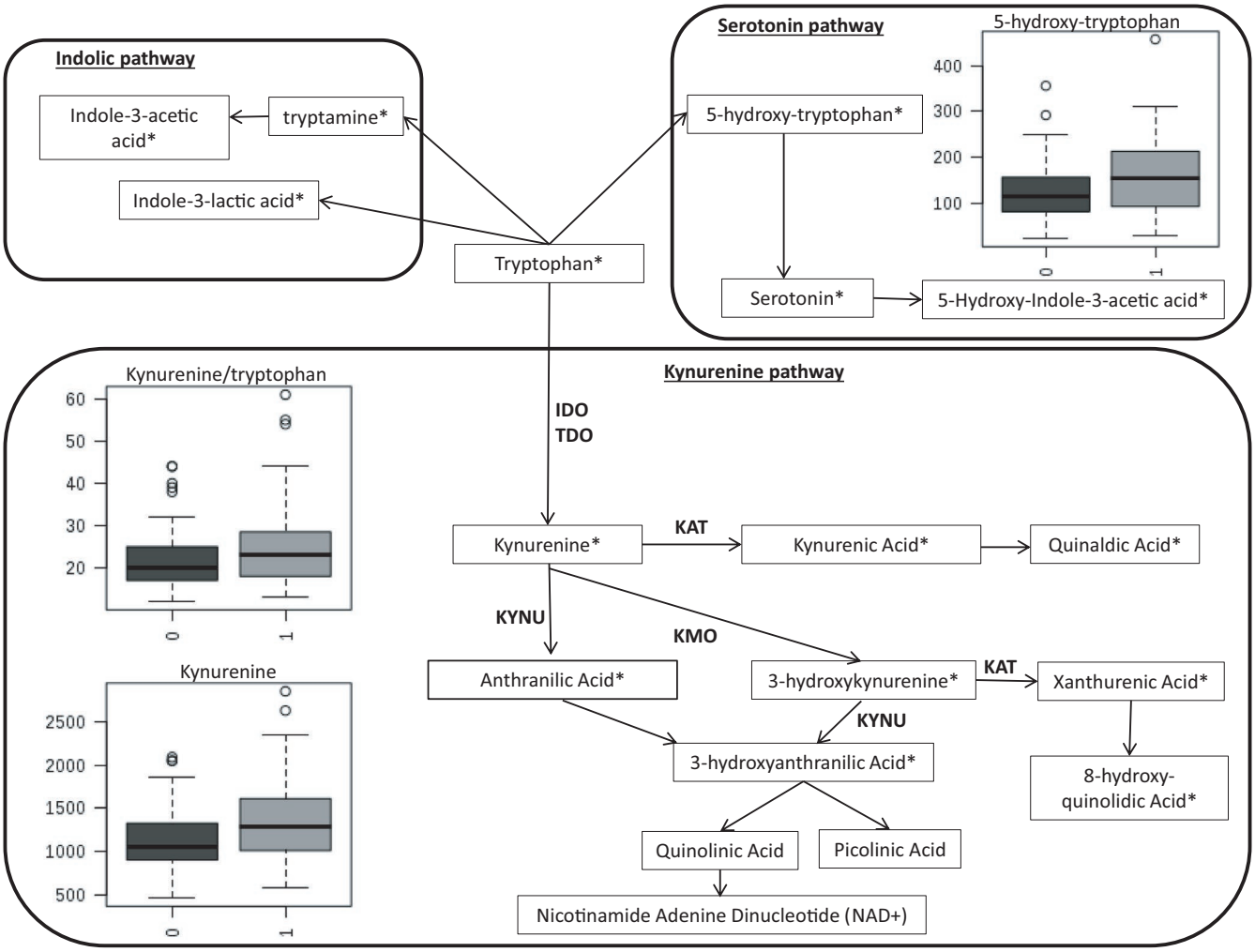


**FIGURE 3** Diagram of metabolites from the tryptophan pathway. Quantified metabolites are indicated with a star (*), and histograms indicate metabolite concentrations (nmol/L) and ratio significantly increased after 1 month of treatment in the exploratory cohort (5-hydroxy-tryptophan $p = 0.02$, kynurenine $p = 0.0005$, and kynurenine/tryptophan $p = 0.02$). Involved enzymes are: IDO, Indoleamine 2,3-Dioxygenase; TDO, Tryptophan 2,3-Dioxygenase; KAT, kynurenine aminotransferase; KYNU, Kynureninase; KMO, kynurenine monooxygenase

cohort. The association of a dysregulated KP and obesity has been well-established both through metabolomics[37] and specific targeted analyses.[30] The main putative mechanisms involve oxidative stress and chronic low-grade inflammation with secretion of diverse pro-inflammatory cytokines that could lead to increased IDO activity, and, consequently, a higher kynurenine/tryptophan ratio. As the KP interacts dynamically with the immune system, it is considered the crossroad between psychiatric pathology, drug exposure, and WG side effects. Interestingly, a recent study has tested the effect of benserazide, an inhibitor of peripheral kynurenine metabolism, on metabolic parameters in 10 mice exposed to olanzapine, an antipsychotic drug with a high risk of WG.[38] It was shown that benserazide attenuates the olanzapine-induced excessive WG after 71 days of olanzapine exposure, and improved other metabolic parameters, such as cholesterol, triglycerides, and glycemia. Targeting this pathway for prevention and treatment of psychotropic drug-induced metabolic syndrome was therefore proposed.

Our metabolomic analysis, which covers a list of 10 acylcarnitines, showed a significant increase in short chain propionylcarnitine and a decrease in intermediate as well as long chain acylcarnitines, namely decanoylcarnitine, octanoylcarnitine, lauroylcarnitine, oleylcarnitine, and hexanoylcarnitine after 1 month of treatment. These changes are consistent with recently published studies.[39–43] Acylcarnitines are organic compounds containing a fatty acid. They have a central role in transporting fatty acids across the mitochondrial inner membrane during beta-oxidation, and are implicated in energy production and cellular homeostasis pathways. Their perturbations confirm mitochondrial dysfunction and disrupted fatty acid beta-oxidation during psychotropic treatment,[42,43] as well as a potential dysfunction of enzymes involved in synthesis of

short-chain acylcarnitines (carnitine acetyltransferase) and medium and long chain acylcarnitines (carnitine palmitoyltransferase 1 or CPT1).[41] Our results show a link between WG over 3 months of treatment and a decrease in hexanoylcarnitine level. Also known as C6, it promotes the transport of medium-chain fatty acids into mitochondria. Its decrease, potentially explained by a dysfunction of CPT1 and a reduction in lipolysis, could result in a decrease of beta-oxidation and, consequently, to an imbalance in energy homeostasis in favor of fatty acids, promoting lipid storage in adipocytes and hepatocytes, and ultimately WG.[44] In light of our results, acylcarnitines, and especially hexanoylcarnitine, might be useful indicators of weight changes in psychiatric patients under WG-inducing psychotropic drugs.

A significant decrease in biliverdin level was found after 1 month of treatment. Biliverdin is a heme metabolite, produced under action of a cytoprotective enzyme, the heme oxygenase (HO).[45] The isoform HO-1 has been shown to be inducible by various stimuli. Biliverdin is then rapidly transformed in bilirubin via biliverdin reductase. Of note, biliverdin can be regenerated by reactive oxygen species (ROS) reactions with bilirubin. Different properties have been recognized in biliverdin and bilirubin, including antioxidant properties. One can presume that different mechanisms, alone or combined, can result in decreased biliverdin levels after WG-inducing psychotropic drug use as an inhibition of HO-1, a higher activation of biliverdin reductase, or an inhibition of regeneration by ROS reactions. We also show that short-term decrease in biliverdin is associated with higher WG over 3 months of treatment. Interestingly, HO-1 expression and activity have been shown to be downregulated in obesity, leading to excessive oxidative stress with impairment of the detoxification mechanism within adipocytes, adipogenesis, and chronic inflammation state.[46] This disruption could also contribute to the development of other metabolic disturbances, such as insulin resistance or vascular dysfunction. Chronic upregulation of HO is recognized as having a beneficial role in weight regulation, reducing adipocyte terminal differentiation, lipid accumulation, and excess of inflammatory molecules.[47] Altogether, this evidence could lead to additional new mechanistic hypotheses of psychotropic drug-induced WG, biliverdin, and HO system being central for early prediction of WG under treatment. However, it needs to be validated with another method to confirm those exploratory findings.

Some limitations of the present study must be mentioned. Metabolome expression could be influenced by physiological state. As the first confounding factor in line, samples were drawn under fasting conditions. However, the nutritional state of the subjects between the two blood samplings in the same individual could be a source of variation that was not taken into account, as this parameter was not monitored in this study. Even if study subjects were carefully selected, as detailed in the Methods section, another unknown confounding factor could also affect metabolome expression, such as other physiological or pathological states, or environmental exposure. A large panel of WG-inducing psychotropic drugs were studied, allowing identification of common metabolomic effects, but preventing the differentiation of specific effects from each type of psychotropic drug. Exploratory analyses of targeted metabolomic results were conducted on the largest subgroup of patients treated with one drug (olanzapine, $n = 14$). No differences were observed in metabolite profiles between pre- and post-treatment in this subgroup (data not shown). Different drugs could involve different mechanisms of metabolic disorders; future studies should therefore include a larger number of patients to allow for the analysis of the effects of each drug separately. On the other hand, grouping patients under different treatments could generate valuable metabolomic results in the objective of identifying a predictive fingerprint of WG independent of the prescribed drug, which could be a relevant biomarker used in personalized medicine. The majority of included patients had current or previous treatment with WG-inducing psychotropic drugs, such medications possibly influencing the metabolomic profile.[48] However, for drug-naïve patients, no difference was observed in their baseline metabolite profile. Furthermore, already-treated patients are representative of the majority of the psychiatric population, therefore reflecting the clinical validity of our cohort.

The large set of metabolomic variables examined, combined with the longitudinal design of the study, allows for consideration of early modifications in the metabolic pattern. Moreover, some of our metabolomic results were confirmed by a specific and validated quantitative analysis of both cohorts that were studied at a temporally different period. In addition, therapeutic drug monitoring of all patients allowed for the exclusion of false negatives (i.e., the absence of modifications of weight and/or metabolomic pattern caused by poor adherence or nonadherence to treatment). With this methodology, we showed that metabolomics could permit the identification of early blood markers associated with WG in treated psychiatric patients. Our current results should be seen as hypothesis-generating ones that need to be confirmed in a larger cohort. Moreover, those findings could be linked with other levels of the cellular regulation process and molecular networks, in order to connect multi-omics measurement.[49] This integrative biology objective would provide better mechanistic comprehension and prediction of WG after exposure to psychotropic drug-induced WG, and improve personalized medicine.

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## CONFLICT OF INTEREST

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## AUTHOR CONTRIBUTIONS

M.L. wrote the manuscript. CB.E. designed the research. M.L., J.S., B.H., C.D., M.A., A.VG., P.C., D.A., A.T., and CB.E. performed the research. M.L., and M.G. analyzed the data. A.T. and D.A. contributed new reagents/analytical tools.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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