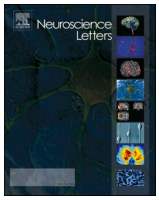
Neuroscience Letters 831 (2024) 137791

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Minireviews

Evidence from preclinical and clinical metabolomics studies on the antidepressant effects of ketamine and esketamine

Daniele Cavaleri [a], Ilaria Riboldi [a], Cristina Crocamo [a], Giuseppe Paglia [a], Giuseppe Carrà [a,b], Francesco Bartoli [a,*]

[a] Department of Medicine and Surgery, University of Milano-Bicocca, Via Cadore 48, 20900 Monza, Italy
[b] Division of Psychiatry, University College London, 149 Tottenham Ct Rd, London W1T 7NF, United Kingdom

ARTICLE INFO

Keywords:
Biomarkers
Esketamine
Ketamine
Metabolomics
Precision psychiatry
Treatment-resistant depression

ABSTRACT

The antidepressant effects of ketamine and esketamine are well-documented. Nonetheless, most of the underlying molecular mechanisms have to be uncovered yet. In the last decade, metabolomics has emerged as a useful means to investigate the metabolic phenotype associated with depression as well as changes induced by antidepressant treatments. This mini-review aims at summarizing the main findings from preclinical and clinical studies that used metabolomics to investigate the metabolic effects of subanesthetic, antidepressant doses of ketamine and esketamine and their relationship with clinical response. Both animal and human studies report alterations in several metabolic pathways – including the tricarboxylic acid cycle, glycolysis, the pentose phosphate pathway, lipid metabolism, amino acid metabolism, the kynurenine pathway, and the urea cycle – following the administration of ketamine or its enantiomers. Although more research is needed to clarify commonalities and differences in molecular mechanisms of action between the racemic compound and its enantiomers, these findings comprehensively support an influence of ketamine and esketamine on mitochondrial and cellular energy production, membrane homeostasis, neurotransmission, and signaling. Metabolomics may thus represent a promising strategy to clarify molecular mechanisms underlying treatment-resistant depression and related markers of clinical response to ketamine and esketamine. This body of preclinical and clinical evidence, if further substantiated, has the potential to guide clinicians towards personalized approaches, contributing to new paradigms in the clinical management of depression.

1. State of the art

The N-methyl-d-aspartate receptor (NMDAR) antagonist ketamine is a dissociative drug used not only as an anesthetic and analgesic agent but also as a recreational substance [1,2]. Nevertheless, it was not until the late 1990s that some studies started suggesting that a single, subanesthetic dose of ketamine has antidepressant properties [3–6].

Several mechanisms have been identified as putatively responsible for the antidepressant effect of ketamine along with the non-competitive voltage-dependent NMDAR inhibition, such as the involvement of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and its signaling cascade [7], the increase in proteins required for formation, maturation, and function of new spines [8], and the modulation of monoaminergic neurotransmission [4].

Ketamine is available not only as a racemic mixture ((R,S)-ketamine) but also as its isolated enantiomers esketamine ((S)-ketamine) and arketamine ((R)-ketamine). Esketamine, which has an affinity for NMDARs threefold to fourfold higher than ketamine, has been shown to be at least as effective as racemic ketamine in decreasing depressive symptoms [9], while arketamine, despite the reported more favorable tolerability profile, failed to show a significant antidepressant effect in the most recent clinical studies [10]. Although the use of racemic ketamine in depression is still off-label, in 2019 a nasal spray formulation of esketamine received regulatory approval by both the United States (US) Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as an add-on option to oral selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs) for the management of treatment-resistant depression (TRD) [11].

Notwithstanding their proven efficacy and their putative mechanisms of antidepressant action, metabolic features likely underlying the antidepressant properties of ketamine and esketamine have yet to be

* Corresponding author at: Department of Medicine and Surgery, University of Milano-Bicocca, via Cadore 48, 20900 Monza, Italy.
E-mail address: francesco.bartoli@unimib.it (F. Bartoli).

https://doi.org/10.1016/j.neulet.2024.137791
Received 15 March 2024; Received in revised form 15 April 2024; Accepted 23 April 2024
Available online 24 April 2024

fully understood [4]. Indeed, albeit hundreds of candidate biomarkers have been examined in relation to response to ketamine or esketamine, a recent systematic review showed no consistent association between baseline blood-based biomarkers and treatment response [12]. In recent years, metabolomics has emerged among the most useful and advanced approaches to investigate molecular mechanisms underlying mental disorders as well as to predict individual variations in drug response phenotypes [13,14].

Metabolomics, providing a global analysis of all metabolites found within a specific biological sample through a simultaneous measurement of plentiful molecules with molecular weight < 1500 Da [15], offers a quantifiable and dynamic readout of the biochemical state of the subject. Therefore, pharmacometabolomics may represent a powerful means to inform our understanding of the pathogenesis of depression, identifying biomarkers for diagnosis and prognosis, and guiding the development of personalized therapeutic interventions, considering also the influences of environment and concomitant pharmacological therapies [14,16,17]. With regard to treatment with ketamine and esketamine, preliminary preclinical and clinical evidence has emerged from metabolomics studies in the last decade. Studies using animal models of depression have identified several metabolic mechanisms potentially contributing to the antidepressant action of ketamine and esketamine [18–29], and similar clues have emerged from human studies [20,30–33].

The aim of this mini-review is thus to synthesize the main findings from studies based on metabolomics which investigated the metabolic effects of ketamine and esketamine as well as relevant candidate biomarkers of clinical response [34,35], discussing their limitations and future directions.

2. Evidence from animal studies

Animal studies regarding the effects of subanesthetic doses of racemic ketamine or its enantiomers on the metabolome provide valuable insights into the drugs' biomolecular effects on energy, lipid, and amino acid (AA) metabolisms. A list of the main metabolisms influenced by the administration of ketamine or its enantiomers in animals, with relevant references, is provided in Table 1.

2.1. Energy metabolism

Energy metabolism seems the domain most affected by ketamine administration, with the involvement of multiple metabolic pathways and cellular processes. In mice, an alteration of the tricarboxylic acid (TCA) cycle can be observed already 2 h after a single intraperitoneal esketamine 3 mg/kg injection, with higher thiamine pyrophosphate, acetyl-coenzyme A (acetyl-CoA), and succinate as well as lower fumarate, malate, and citrate/acetyl-CoA ratio relative to saline solution [29]. Malate levels are reportedly lower also 14 h upon esketamine injection [29] and 24 h upon arketamine administration [24], whilst the succinate/fumarate ratio is lower after 14 h [29]. These changes in TCA cycle metabolites and metabolite ratios are consistent with ketamine-induced NMDAR blockade, which results in a decreased Ca^{2+} flux into cells and mitochondria [36]. Since several enzymes of the TCA cycle (including succinate dehydrogenase) are regulated by Ca^{2+} [37], ketamine may influence these enzymes and ultimately the TCA cycle balance. Other metabolites directly or indirectly involved in the TCA cycle may also serve as markers of ketamine response: for instance, fumarate, methylmalonate, and 2-ketoisovalerate have been suggested to represent stable and consistent hippocampal biomarkers of response to esketamine between 2 and 72 h upon administration [29]. Furthermore, time-dependent alterations in energy equivalents and relevant ratios following esketamine injection have been observed: adenosine triphosphate (ATP), guanosine triphosphate (GTP), and the GTP/guanosine diphosphate (GDP) ratio seem increased after 2 h and decreased after 24 h. In particular, elevated GTP levels 2 h after ketamine injection are in

Table 1
List of the main metabolisms/pathways influenced by the administration of ketamine or its enantiomers in animals and humans with relevant references.

	Preclinical studies	Clinical studies
Tricarboxylic acid cycle	Lian et al., 2018 [24] Weckmann et al., 2014 [29]	Rotroff et al., 2016 [32]
Glycolysis	Weckmann et al., 2014 [29]	Rotroff et al., 2016 [32]
Pentose phosphate pathway	Moaddel et al., 2022 [20] Chen et al., 2020 [22] McGowan et al., 2018 [25]	–
Energy equivalents synthesis	Weckmann et al., 2017 [26] Weckmann et al., 2014 [29]	–
AMPK pathway	Weckmann et al., 2017 [26]	–
Purine metabolism	Chen et al., 2020 [22] McGowan et al., 2018 [25] Weckmann et al., 2017 [26] Weckmann et al., 2014 [29]	–
Pyrimidine metabolism	McGowan et al., 2018 [25] Weckmann et al., 2014 [29]	–
Glycerophospholipid metabolism	Liu et al., 2023 [18] Zhou et al., 2023 [19] Moaddel et al., 2022 [20] Chen et al., 2020 [22]	Moaddel et al., 2022 [20] Moaddel et al., 2018 [31] Rotroff et al., 2016 [32] Villasenor et al., 2014 [33]
Sphingolipid metabolism	Liu et al., 2023 [18] Zhou et al., 2023 [19] Moaddel et al., 2022 [20]	Moaddel et al., 2022 [20] Singh et al., 2022 [30] Moaddel et al., 2018 [31] Rotroff et al., 2016 [32] Villasenor et al., 2014 [33]
Fatty acid metabolism	Zhou et al., 2023 [19]	Singh et al., 2022 [30] Rotroff et al., 2016 [32]
Glutamate/GABA-glutamine cycle	Weckmann et al., 2019 [23] Lian et al., 2018 [24] McGowan et al., 2018 [25]	Singh et al., 2022 [30] Rotroff et al., 2016 [32]
Glycine, serine, and threonine metabolism	Witkin et al., 2017 [27] Weckmann et al., 2014 [29]	Rotroff et al., 2016 [32]
Phenylalanine metabolism	–	Rotroff et al., 2016 [32] Villasenor et al., 2014 [33]
Tryptophan metabolism	Moaddel et al., 2022 [20]	Moaddel et al., 2022 [20] Singh et al., 2022 [30] Moaddel et al., 2018 [31] Rotroff et al., 2016 [32]
Urea cycle	Lian et al., 2018 [24]	Moaddel et al., 2018 [31] Rotroff et al., 2016 [32]

AMPK = adenosine monophosphate-activated protein kinase; GABA = γ-aminobutyric acid.

line with the aforementioned higher concentrations of succinate, which is produced from succinyl-CoA in the TCA cycle with the concomitant generation of GTP [29]. Moreover, increased phosphorylated adenosine monophosphate (AMP)-activated protein kinase (pAMPK) levels and pAMPK/AMPK ratio assessed 24 h upon esketamine administration [26], which are likely determined by the reduction in the ATP/ADP ratio, further support an enhanced catabolic activity to restore ATP reserves [38]. Notably, ATP, ADP, and GTP, all seem to correlate with forced swim test floating time at the 24-hour time point [26]. Taken together, these alterations may suggest that higher levels of energy equivalents are initially produced upon ketamine administration but are followed by a decrease after 24 h, returning to normal levels at the 72-hour time point. This likely reflects the dynamic interplay between ketamine-induced initial metabolic response, intermediate compensatory mechanisms, and the eventual "*recovery*" with restoration of metabolic homeostasis. In all, these findings represent evidence for an important role of mitochondrial energy metabolism in ketamine's mechanisms of action.

Again concerning energy production, changes in the levels of several metabolites of cytoplasm-based glycolysis/gluconeogenesis and pentose phosphate pathway in mouse hippocampus upon esketamine administration have been described. A number of early intermediates of these pathways (glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, glyceraldehyde-3-phosphate, and dihydroxy-acetone-phosphate) are significantly lower in mice 14 h upon a single esketamine injection compared to those receiving saline solution [29]. This evidence, together with reported alterations in relevant metabolite ratios (mirroring glycolysis enzymatic activity), suggests a reduced glycolytic flux, indicating a decrease in glucose utilization or a shift away from glycolysis towards alternative pathways. This is consistent with the ketamine-induced NMDAR blockade that has been reported to cause glycolysis deficits [39,40]. However, higher 3-phosphoglycerate and altered ratios of downstream metabolites – indicating abnormal enzymatic activity – may also point towards an increase in the conversion rate of glyceraldehyde-3-phosphate to downstream glycolysis metabolites induced by esketamine. Interestingly, no significant differences in the levels of pyruvate – the end-product of glycolysis – between esketamine and saline groups of mice have been found [29]. Nonetheless, lower urinary excretion of pyruvate was observed in macaques after a single ketamine 10 mg/kg injection [28], possibly suggesting the existence of mechanisms aimed at maintaining cellular energetics homeostasis in response to ketamine. Also, an effect of ketamine on the pentose phosphate pathway has been reported, showing altered levels of pathway intermediates ribose-5-phosphate and ribulose-5-phosphate as well as of nicotinamide adenine dinucleotide phosphate ($NADP^+$) and its reduced form ($NADPH{\cdot}H^+$) [20,22,25]. However, the notable heterogeneity in experimental conditions across the available studies precludes a clear interpretation of findings.

Relating to this, several nucleosides and nucleotides involved in purine metabolism are altered in prefrontal cortex, hippocampus, and striatum of ketamine-treated mice and rats at different time points [22,25,26,29], suggesting an effect of the drug on purine metabolism, which is known to be altered in depression [41]. In addition, ketamine alters pyrimidine metabolism, with changes in the levels of compounds such as deoxyuridine diphosphate and cytosine in mouse prefrontal cortex and hippocampus [25,29].

Some other compounds relating to energy metabolism, such as creatine, phosphocreatine, and inosine are also downregulated in the hippocampus of mice and rats after a single dose of ketamine [24,27], further suggesting an influence on bioenergetics.

2.2. Lipid metabolism

Lipid metabolism seems remarkably impacted by ketamine. First, glycerophospholipids seem downregulated by ketamine in almost every mouse brain subregion, with a reduction in several compounds, especially phosphatidylinositol in the prefrontal cortex and the hippocampus [18,19], phosphatidylcholines in the prefrontal cortex and the hypothalamus [18–20], and phosphatidylinositol phosphate, phosphatidylinositol, and phosphatidylethanolamine in the hippocampus [19]. Alterations of glycerophospholipid metabolism are also seen in prefrontal cortex, hippocampus, and striatum of rats administered with ketamine 30 mg/kg for 10 consecutive days, 24 h after the last injection [22]. Also, sphingolipid metabolism is altered by racemic ketamine and esketamine across different mouse brain regions, with sphingomyelins most significantly changed in the olfactory bulb [18], the hippocampus, and the prefrontal cortex [19], and ceramides in the prefrontal cortex [18,19] and hippocampus [20]. However, the main alterations in sphingolipid metabolism are observed in the pallidal, also with spatial differences within the pallidal itself, with most changes detected in the medial and ventral parts [18].

These observations comprehensively suggest that esketamine antidepressant effects may occur through regional-specific regulation of membrane lipid metabolism [18,19]. Similar alterations upon ketamine treatment are likewise seen in mouse plasma, where some phosphatidylcholines, sphingomyelins, ceramides, and cholesterol esters are increased following treatment [20]. Esketamine also seems to hold the ability to normalize the levels of most fatty acyls and to increase acylcarnitines in the hippocampus and in the prefrontal cortex [19], possibly implying enhanced transportation of fatty acids into the mitochondria for β-oxidation for the benefit of energy production. Since all these compounds are closely related to mitochondrial function, participating in energy metabolism, membrane dynamics, and cellular signaling pathways, these findings add to the aforementioned evidence on the effects of ketamine on mitochondrial and cellular energetics.

2.3. Amino acid metabolism

Ketamine affects AA levels across various metabolisms. In mouse hippocampus, a tendency towards increased levels of AA neurotransmitters γ-aminobutyric acid (GABA) and glutamate can be seen 2 h upon a single esketamine 3 mg/kg injection [23], consistently with previous findings from non-metabolomics investigations [42,43]. Conversely, GABA, glutamate, and glutamine have been found to be reduced both 14 h after a single esketamine 3 mg/kg injection [23] as well as 24 h after a single arketamine 10 mg/kg injection [24]. This pattern appears consistent with the hypothesis of a time dependence of the effects of subanesthetic ketamine on glutamate/GABA-glutamine cycling: the initial increase in hippocampal GABA and glutamate may not be sustained over time but diminish or even reverse in the subsequent hours [42]. However, GABA levels and the GABA/glutamate ratio seemingly increase again at 72 h [23]. Remarkably, the correlations of GABA, glutamate, and glutamine levels with the behavioral forced swim test floating time after esketamine administration [23] suggest a relationship between their concentrations and the drug's antidepressant effects. Of note, besides the glutamate/GABA-glutamine cycle, the levels of aspartate – which acts as an excitatory neurotransmitter similarly to glutamate – also seem reduced at 24 h [24]. Ketamine seems to influence AA neurotransmitter metabolites also in the long term, since a single prophylactic dose (30 mg/kg) produces durable changes in their levels in the prefrontal cortex and the hippocampus of stressed mice. Indeed, increased inhibitory neurotransmitter metabolites (alanine, GABA, and taurine) and decreased excitatory neurotransmitter metabolites (serine, tyrosine, and phenylalanine) all are still detectable 2 weeks upon the prophylactic administration [25]. The main exception to this is the excitatory neurotransmitter glutamate, which is increased [25]. It could be speculated that, since glutamate is also a precursor of GABA, high

glutamate levels are related to an increase in GABA [44]. Nevertheless, as a whole, this bulk of evidence suggests that prophylactic ketamine may increase inhibitory tone in the brain following its administration under stressful circumstances, resulting in long-lasting protection. Interestingly, these alterations are not seen in ketamine-treated mice not exposed to stress [25], possibly implying that the interaction between stress and ketamine is crucial to determine long-lasting metabolic changes that affect stress-related behavior. This is consistent with the hypothesis that ketamine increases stress resilience by regulating GABA and glutamate neurotransmission [45].

However, not only AAs directly involved in neurotransmission seem influenced by ketamine in rodents, whether in brain tissues or plasma [20,27,29]. For instance, ketamine can alter glycine, serine, and threonine metabolism in the hippocampus of mice and rats already after 2 h [27,29], notably with increased serine after a single injection of esketamine 3 mg/kg [29]. Serine can be converted into glycine but also into D-serine, an important co-agonist of the NMDAR. Also, considering that serine can be synthesized from 3-phosphoglycerate, an intermediate of glycolysis, and is linked to the TCA cycle [46], its increase may further point towards an alteration in energy metabolism. Furthermore, tryptophan (TRP) and its product serotonin both seem altered by ketamine administration in brain and plasma of mice, but in an unclear way over time [20].

Ketamine also increases the mouse hippocampal concentrations of urea 24 h upon administration [24], suggesting accelerated AA catabolism.

Interestingly, the aforementioned AAs – along with other non-amino acidic compounds – seem differentially influenced when 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX), a synthetic AMPAR selective antagonist, is co-administered: indeed, while some AAs (glycine, alanine, glutamine, and aspartate) are changed irrespectively of co-administration of NBQX, other metabolites involved in energy metabolism, cellular signaling, and oxidative stress metabolism that are altered upon arketamine treatment (phosphate, urea, GABA, creatine, malate, galactinol, inosine, and aminomalonate) remain unchanged when NBQX is concomitantly administered [24]. This implies that ketamine's mechanisms of action involve both AMPAR-dependent and AMPAR-independent pathways.

3. Evidence from human studies

Although with an intrinsic, unavoidable diversity, several findings from research in human subjects are consistent with preclinical evidence, showing effects of ketamine and its enantiomers on energy, lipid, and AA metabolisms. A list of the main metabolisms influenced by the administration of ketamine or esketamine in humans, with relevant references, is provided in Table 1.

3.1. Energy metabolism

Although human metabolomics studies provide limited data concerning biomolecules involved in energy metabolism, the few available align with the rich evidence emerging from animal investigations. In people with TRD, increased citrate and concurrently decreased lactate are seen in plasma 2 h after ketamine 0.5 mg/kg infusion [32]. This suggests an increased mitochondrial activity with consequent reduced lactate accumulation through anaerobic glycolysis. In addition, increased availability of mannose and fructose is observed 2 h upon ketamine infusion [32], possibly reflecting a reduction in their metabolism through glycolysis. Taken together, these findings may imply that ketamine favors aerobic metabolism and TCA cycle activity over anaerobic glycolysis to produce more energy equivalents within few hours upon ketamine administration. This is consistent with preclinical evidence of increased concentrations of succinate, ATP, and GTP after 2 h [26,29].

3.2. Lipid metabolism

In line with results emerging from animal investigations, clinical studies underscore that lipid metabolism might hold a crucial role in ketamine's antidepressant action in TRD.

Several phosphatidylcholines are altered (mostly increased) in human plasma upon treatment with ketamine, not only in people with TRD [30] but also in healthy individuals [20]. Notably, the levels of phosphatidylcholines – and particularly lysophosphatidylcholines – are generally higher in responders than in non-responders 4 h after ketamine administration [31,33], though substantially restored to baseline levels within 24 h [31]. Phosphatidylcholine concentrations also correlate with clinical response, increasing as depressive symptoms decrease 2 h after ketamine treatment [32]. Interestingly, these patterns seem to differ according to concomitant drug treatment: responders maintained on lithium – but not those on valproate – show changes in the concentrations of lysophosphatidylcholines and lysophosphatidylethanolamines [33]. Very similar patterns have also been observed in patients receiving placebo [33], suggesting that the differences may be linked more to the basal treatment with valproate or lithium rather than to ketamine. In all, findings on phospholipids may be indicative of alterations in the composition of membranes (of which they are main constituents), signaling, energy metabolism, and unfolded protein response [47].

Ketamine also influences sphingolipid metabolism [20,30–33]: a number of sphingomyelins is indeed altered by ketamine relative to placebo [30,31,33], with some increasing within 24 h after ketamine administration in both TRD [30,31] and healthy subjects [20]. Sphingomyelins seem to increase as depressive symptoms decrease [32], though not consistently across studies [31]. In addition, ceramides, cholesteryl esters, and triglycerides are increased in plasma after ketamine administration, with ceramides and triglycerides also significantly correlating with symptom improvement in TRD [30]. Furthermore, ketamine and esketamine (but not placebo) induce notable alterations in several acylcarnitines in plasma of people with TRD within 2 h post-infusion [32]. In particular, a decrease in short- and medium-chain acylcarnitines and an increase in long-chain ones within 40 min, followed by a subsequent increase in short-chain ones, have been described [30]. Remitters show a more prominent decrease in short-chain acylcarnitines at 40 min [30] and, as depressive symptoms improve upon treatment with ketamine, acylcarnitines levels increase [30,32]. This further supports the hypothesis that ketamine alters fatty acid transport into mitochondria for energy production through β-oxidation. Overall, findings from human studies investigating the influence of antidepressant doses of ketamine and esketamine on lipid metabolism align with preclinical data to indicate that these drugs have an impact on mitochondrial and cellular energy metabolism, cell membrane, and signaling pathways.

3.3. Amino acid metabolism

Consistently with preclinical evidence, subanesthetic doses of ketamine reportedly influence the plasmatic concentrations of several AAs in humans [20,30,32,33], possibly acting on enzymes involved in their metabolism but also on their transport across cell membranes or their uptake by tissues. Similar to animal studies, research in people with TRD points towards alterations in AAs involved in neurotransmission as well as in those not directly related to this.

First, ketamine seems to alter AA neurotransmitters GABA and glutamate in human plasma in the short term in a way that is coherent with what was found in mouse brain [23,42,43]. Increased GABA [30] and glutamate [32] are observed within 2 h post-ketamine infusion in TRD. Moreover, consistently with the drop in glutamate levels seen in mouse brain 14–24 h upon injection [23,24], subjects with TRD who positively respond to ketamine show a greater reduction in glutamate levels compared to non-responders [30]. This further substantiates the

putative time dependence of the effects of subanesthetic ketamine on glutamate/GABA-glutamine cycling [42].

Second, phenylalanine metabolism seems influenced by ketamine, since a relative increase in its plasma levels has been observed in people with treatment-resistant bipolar depression after a single ketamine injection, regardless of clinical response [33]. Moreover, plasmatic tyrosine, synthesized from phenylalanine by phenylalanine hydroxylase with the involvement of cofactor tetrahydrobiopterin [48], is reduced in TRD after esketamine dosing [32]. Thus, it can be argued that ketamine modulates phenylalanine hydroxylase or that it increases tyrosine conversion to dopamine, norepinephrine, and epinephrine. In addition, the levels of phenyllactic acid, a product of phenylalanine catabolism, are lower in responders to ketamine compared to non-responders in treatment-resistant bipolar depression [33]. This further hints at an influence of the drug on phenylalanine metabolism and catecholamine synthesis possibly underlying ketamine's antidepressant effects [49].

Third, ketamine apparently alters TRP metabolism. TRP decreases 2 h after treatment [32] and until 24 h, with a later recovery [20]. Interestingly, there seems to be a discrepancy in TRP concentrations between plasma and cerebrospinal fluid, with increased levels in the former while decreased in the latter [20]. As regards TRP catabolism and the kynurenine (KYN) pathway, which is known to be altered in both unipolar and bipolar depression [50–52], responders to ketamine have lower plasma KYN levels and KYN/TRP ratio, as well as higher anthranilic acid and picolinic acid levels at 230 min, with the difference in the KYN/TRP ratio maintained up to 24 h later [31]. Moreover, KYN plasmatic concentrations [30] and the KYN/TRP ratio [32] both seem to correlate with depressive symptom improvement. Other molecules derived from TRP metabolism (indole-3-acetate, indole-3-lactate) are putatively decreased after treatment with ketamine or esketamine [32].

Fourth, as suggested by animal studies [27,29], glycine, serine, and threonine metabolism is influenced by ketamine: decreased plasmatic threonine is observed 2 h upon esketamine treatment in TRD [32], with potential consequences on neurotransmission and energy metabolism.

Finally, ketamine may exert its antidepressant action by influencing the urea cycle and nitric oxide synthesis, as suggested by the higher arginine plasmatic levels consistently seen in responders 2–4 h after treatment [31,32]. The supposed urea cycle involvement is further substantiated by the increase in plasma ornithine and citrulline parallel to depressive symptom improvement [32].

4. Implications and limitations of the existing literature and future directions

Despite this body of preclinical and clinical studies, several limitations hinder the translation of findings into clinical implications.

First, evidence derived from human studies, generally based on peripheral blood samples, has an intrinsically diverse nature from that from animal studies, mainly analyzing brain tissue. Second, the findings, especially those from human studies, suffer from limited sample sizes, requiring replication in larger cohorts. Moreover, there is considerable variability in the timing of metabolomic assessments relative to drug administration across available studies. Likewise, studies should consider the effects of repeated ketamine dosing in people with TRD over extended periods. Besides, the impact of concomitant maintenance treatments warrants further exploration, as different underlying pharmacotherapy (serotonergic antidepressants, lithium, valproate) may differently influence metabolic alterations and treatment outcomes [32,53]. In addition, the complexity of data interpretation is increased by the different agents tested, since most studies focused on ketamine, other investigated esketamine, and a few tested arketamine. In particular, the molecular mechanisms of action of the racemic compound and of its enantiomers seem to involve common metabolisms, however only one study in humans [32] – and none in animal models – have assessed both racemic ketamine and esketamine in the same population. Hence, further direct comparisons are needed for a deeper exploration of relevant commonalities and differences. To further broaden our knowledge, more information on the association of metabolic changes with improvements in depressive symptoms needs to be collected, focusing on those compounds – such as phosphatidylcholines, sphingomyelins, acylcarnitines, KYN/TRP ratio, ornithine, and citrulline – whose alterations after treatment have been significantly correlated with depressive symptoms improvement [30,32]. Moreover, no satisfactory baseline predictor of treatment response has been identified yet. It should be also clarified whether ketamine and esketamine metabolic effects may vary according to the baseline stress/depressive conditions [45].

Overall, metabolomics studies on subanesthetic, antidepressant doses of ketamine and its enantiomers are still at a hypothesis-generating level. Nonetheless, metabolomics has already provided many interesting and valuable insights into the mechanisms of antidepressant action of ketamine and esketamine that cannot be observed through classical biochemical assays. This body of evidence, if further substantiated and validated, has the potential to recognize candidate biomarkers for diagnosis and treatment of TRD, ultimately guiding clinicians towards personalized approaches and contributing to new paradigms in the clinical management of the disorder [34,54]. Finally, metabolomics may provide valuable insight into the neurobiology of TRD itself, driving the identification of novel therapeutic targets and the development of next-generation antidepressants. Notably, the understanding of molecular mechanisms involved in response to treatment with ketamine and its enantiomers may provide a basis for further drug discovery. For instance, the glutamatergic system has already attracted attention as target for the development of new antidepressants. In particular, group II metabotropic glutamate (mGlu) receptors are of interest due to their modulatory role in glutamatergic transmission [55,56]. Preliminary evidence indicates that mGlu2/3 receptor antagonists hold antidepressant effects and relevant underlying mechanisms that resemble those of ketamine, possibly with less side effects [55]. Clinical studies of several mGlu2/3 receptor antagonists (e.g., decoglurant and TS-161) for depression have been or are being carried out [56] and, notwithstanding some controversial results, they seem to hold potential for the development of safer and more efficacious antidepressants.

Additional studies investigating predictors of response and longitudinal metabolome changes amid treatment with ketamine and esketamine are thus needed to refine treatment strategies and to contribute to clinical development for TRD.

5. Role of the funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

6. Significance statement

Identifying molecular mechanisms underlying the antidepressant effects of ketamine and esketamine through metabolomics studies may contribute to the identification of biomarkers, the development of new drugs, and the definition of novel paradigms for precision psychiatry approaches for treatment-resistant depression.

CRediT authorship contribution statement

Daniele Cavaleri: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Ilaria Riboldi:** Writing – review & editing, Investigation, Data curation. **Cristina Crocamo:** Writing – review & editing, Investigation, Data curation. **Giuseppe Paglia:** Writing – review & editing, Validation, Methodology. **Giuseppe Carrà:** Writing – review & editing, Validation, Methodology. **Francesco Bartoli:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgment

None.

References

[1] J. Van Amsterdam, W. Van Den Brink, Harm related to recreational ketamine use and its relevance for the clinical use of ketamine. A systematic review and comparison study, Exp. Op. Drug Safety. 21 (2022) 83–94, https://doi.org/10.1080/14740338.2021.1949454.
[2] L. Li, P.E. Vlisides, Ketamine: 50 Years of Modulating the Mind, Front. Hum. Neurosci. 10 (2016) 612, https://doi.org/10.3389/fnhum.2016.00612.
[3] A. Bahji, G.H. Vazquez, C.A. Zarate Jr., Comparative efficacy of racemic ketamine and esketamine for depression: A systematic review and meta-analysis, J. Affect. Disord. 278 (2021) 542–555, https://doi.org/10.1016/j.jad.2020.09.071.
[4] P. Zanos, T.D. Gould, Mechanisms of ketamine action as an antidepressant, Mol. Psychiatry. 23 (2018) 801–811, https://doi.org/10.1038/mp.2017.255.
[5] C.A. Zarate Jr, J.B. Singh, P.J. Carlson, et al., A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression, Arch. Gen. Psychiatry. 63 (2006) 856–864, https://doi.org/10.1001/archpsyc.63.8.856.
[6] R.M. Berman, A. Cappiello, A. Anand, D.A. Oren, G.R. Heninger, D.S. Charney, J. Krystal, Antidepressant effects of ketamine in depressed patients, Biol. Psychiatry. 47 (2000) 351–354, https://doi.org/10.1016/s0006-3223(99)00230-9.
[7] P. Zanos, R. Moaddel, P.J. Morris, NMDAR inhibition-independent antidepressant actions of ketamine metabolites, Nature. 533 (2016) 481–486, https://doi.org/10.1038/nature17998.
[8] N. Li, B. Lee, R.J. Liu, et al., mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists, Science. 329 (2010) 959–964, https://doi.org/10.1126/science.1190287.
[9] J.B. Singh, M. Fedgchin, E. Daly, et al., Intravenous Esketamine in Adult Treatment-Resistant Depression: A Double-Blind, Double-Randomization, Placebo-Controlled Study, Biol. Psychiatry. 80 (2016) 424–431, https://doi.org/10.1016/j.biopsych.2015.10.018.
[10] S. Koncz, N. Papp, D. Pothorszki, G. Bagdy, (S)-Ketamine but Not (R)-Ketamine Shows Acute Effects on Depression-Like Behavior and Sleep-Wake Architecture in Rats, Int. J. Neuropsychopharmacol. 26 (2016) 618–626, https://doi.org/10.1093/ijnp/pyad050.
[11] E. Mahase, Esketamine is approved in Europe for treating resistant major depressive disorder, Br. Med. J. 367 (2019) l7069, https://doi.org/10.1136/bmj.l7069.
[12] G.C. Medeiros, T.D. Gould, W.L. Prueitt, et al., Blood-based biomarkers of antidepressant response to ketamine and esketamine: A systematic review and meta-analysis, Mol. Psychiatry. 27 (2022) 3658–3669, https://doi.org/10.1038/s41380-022-01652-1.
[13] R.D. Beger, M.A. Schmidt, R. Kaddurah-Daouk, Current Concepts in Pharmacometabolomics, Biomarker Discovery, and Precision Medicine, Metabolites. 10 (2020) 129, https://doi.org/10.3390/metabo10040129.
[14] P.B. Shih, Metabolomics Biomarkers for Precision Psychiatry, Adv. Exp. Med. Biol. 1161 (2019) 101–113, https://doi.org/10.1007/978-3-030-21735-8_10.
[15] G. Paglia, F.M. Del Greco, B.B. Sigurdsson, et al., Influence of collection tubes during quantitative targeted metabolomics studies in human blood samples, Clin. Chim. Acta. 486 (2018) 320–328, https://doi.org/10.1016/j.cca.2018.08.014.
[16] E. Gianazza, M. Brioschi, A. Iezzi, G. Paglia, C. Banfi, Pharmacometabolomics for the Study of Lipid-Lowering Therapies: Opportunities and Challenges, Int. J. Mol. Sci. 24 (2023) 3291, https://doi.org/10.3390/ijms24043291.
[17] G. Paglia, M. Stocchero, S. Cacciatore, et al., Unbiased Metabolomic Investigation of Alzheimer's Disease Brain Points to Dysregulation of Mitochondrial Aspartate Metabolism, J. Proteom. Res. 15 (2016) 608–618, https://doi.org/10.1021/acs.jproteome.5b01020.
[18] G.X. Liu, Z.L. Li, S.Y. Lin, et al., Mapping metabolite change in the mouse brain after esketamine injection by ambient mass spectrometry imaging and metabolomics, Front. Psychiatry. 14 (2023) 1109344, https://doi.org/10.3389/fpsyt.2023.1109344.
[19] C. Zhou, X. Zhao, X. Ma, et al., Effects of (S)-ketamine on depression-like behaviors in a chronic variable stress model: a role of brain lipidome, Front. Cell. Neurosci. 17 (2023) 1114914, https://doi.org/10.3389/fncel.2023.1114914.
[20] R. Moaddel, P. Zanos, C.A. Farmer, et al., Comparative metabolomic analysis in plasma and cerebrospinal fluid of humans and in plasma and brain of mice following antidepressant-dose ketamine administration, Transl. Psychiatry. 12 (2022) 179, https://doi.org/10.1038/s41398-022-01941-x.
[21] T.L. Emmerzaal, L. Jacobs, B. Geenen, et al., Chronic fluoxetine or ketamine treatment differentially affects brain energy homeostasis which is not exacerbated in mice with trait suboptimal mitochondrial function, Eur. J. Neurosci. 53 (2021) 2986–3001, https://doi.org/10.1111/ejn.14901.
[22] F. Chen, Y. Ye, X. Dai, Y. Zheng, S. Fang, L. Liao, Metabolic effects of repeated ketamine administration in the rat brain, Biochem. Biophys. Res. Commun. 522 (2020) 592–598, https://doi.org/10.1016/j.bbrc.2019.11.140.
[23] K. Weckmann, M.J. Deery, J.A. Howard, et al., Ketamine's Effects on the Glutamatergic and GABAergic Systems: A Proteomics and Metabolomics Study in Mice, Mol. Neuropsychiatry. 5 (2019) 42–51, https://doi.org/10.1159/000493425.
[24] B. Lian, J. Xia, X. Yang, et al., Mechanisms of ketamine on mice hippocampi shown by gas chromatography-mass spectrometry-based metabolomic analysis, Neuroreport. 29 (2018) 704–711, https://doi.org/10.1097/WNR.0000000000001020.
[25] J.C. McGowan, C. Hill, A. Mastrodonato, et al., Prophylactic ketamine alters nucleotide and neurotransmitter metabolism in brain and plasma following stress, Neuropsychopharmacol. 43 (2018) 1813–1821, https://doi.org/10.1038/s41386-018-0043-7.
[26] K. Weckmann, M.J. Deery, J.A. Howard, et al., Ketamine's antidepressant effect is mediated by energy metabolism and antioxidant defense system, Sci. Rep. 7 (2017) 15788, https://doi.org/10.1038/s41598-017-16183-x.
[27] J.M. Witkin, S.N. Mitchell, K.A. Wafford, et al., Comparative Effects of LY3020371, a Potent and Selective Metabotropic Glutamate (mGlu) 2/3 Receptor Antagonist, and Ketamine, a Noncompetitive N-Methyl-d-Aspartate Receptor Antagonist in Rodents: Evidence Supporting the Use of mGlu2/3 Antagonists, for the Treatment of Depression, J. Pharmacol. Exp. Ther. 361 (2017) 68–86, https://doi.org/10.1124/jpet.116.238121.
[28] X. Pan, X. Zeng, J. Hong, et al., Effects of Ketamine on Metabolomics of Serum and Urine in Cynomolgus Macaques (Macaca fascicularis), J. Am. Assoc. Lab. Anim. Sci. 55 (2016) 558–564.
[29] K. Weckmann, C. Labermaier, J.M. Asara, M.B. Müller, C.W. Turck, Time-dependent metabolomic profiling of Ketamine drug action reveals hippocampal pathway alterations and biomarker candidates, Transl. Psychiatry. 4 (2014) e481.
[30] B. Singh, S. MahmoudianDehkordi, J.L.V. Voort, et al., Metabolomic signatures of intravenous racemic ketamine associated remission in treatment-resistant depression: A pilot hypothesis generating study, Psychiatry Res. 314 (2022) 114655, https://doi.org/10.1016/j.psychres.2022.114655.
[31] R. Moaddel, M. Shardell, M. Khadeer, et al., Plasma metabolomic profiling of a ketamine and placebo crossover trial of major depressive disorder and healthy control subjects, Psychopharmacol. 235 (2018) 3017–3030, https://doi.org/10.1007/s00213-018-4992-7.
[32] D.M. Rotroff, D.G. Corum, A. Motsinger-Reif, et al., Metabolomic signatures of drug response phenotypes for ketamine and esketamine in subjects with refractory major depressive disorder: new mechanistic insights for rapid acting antidepressants, Transl. Psychiatry. 6 (2016) e894.
[33] A. Villaseñor, A. Ramamoorthy, M. Silva dos Santos, et al., A pilot study of plasma metabolomic patterns from patients treated with ketamine for bipolar depression: evidence for a response-related difference in mitochondrial networks, Br. J. Pharmacol. 171 (2014) 2230–2242, https://doi.org/10.1111/bph.12494.
[34] I. Estrade, A.C. Petit, V. Sylvestre, et al., Early effects predict trajectories of response to esketamine in treatment-resistant depression, J. Affect. Disord. 342 (2023) 166–176, https://doi.org/10.1016/j.jad.2023.09.030.
[35] C.A. Zarate Jr, D.C. Mathews, M.L. Furey, Human biomarkers of rapid antidepressant effects, Biol. Psychiatry 73 (2013) 1142–1155, https://doi.org/10.1016/j.biopsych.2012.11.031.
[36] C. Wang, F. Liu, T.A. Patterson, M.G. Paule, W. Slikker Jr., Relationship between ketamine-induced developmental neurotoxicity and NMDA receptor-mediated calcium influx in neural stem cell-derived neurons, Neurotoxicol. 60 (2017) 254–259, https://doi.org/10.1016/j.neuro.2016.04.015.
[37] B. Glancy, R.S. Balaban, Role of mitochondrial Ca2+ in the regulation of cellular energetics, Biochem. 51 (2012) 2959–2973, https://doi.org/10.1021/bi2018909.
[38] D. Carling, F.V. Mayer, M.J. Sanders, S.J. Gamblin, AMP-activated protein kinase: nature's energy sensor, Nat. Chem. Biol. 7 (2011) 512–518, https://doi.org/10.1038/nchembio.610.
[39] J. Hu, W. Duan, Y. Liu, Ketamine inhibits aerobic glycolysis in colorectal cancer cells by blocking the NMDA receptor-CaMK II-c-Myc pathway, Clin. Exp. Pharmacol. Physiol. 47 (2020) 848–856, https://doi.org/10.1111/1440-1681.13248.
[40] P.C. Guest, K. Iwata, T.A. Kato, et al., MK-801 treatment affects glycolysis in oligodendrocytes more than in astrocytes and neuronal cells: insights for schizophrenia, Front. Cell. Neurosci. 9 (2015) 180, https://doi.org/10.3389/fncel.2015.00180.
[41] F. Bartoli, G. Burnstock, C. Crocamo, G. Carrà, Purinergic Signaling and Related Biomarkers in Depression, Brain Sci. 10 (2020) 160, https://doi.org/10.3390/brainsci10030160.
[42] G.M. Chowdhury, J. Zhang, M. Thomas, et al., Transiently increased glutamate cycling in rat PFC is associated with rapid onset of antidepressant-like effects, Mol. Psychiatry. 22 (2017) 120–126, https://doi.org/10.1038/mp.2016.34.
[43] G.M. Chowdhury, K.L. Behar, W. Cho, M.A. Thomas, D.L. Rothman, G. Sanacora, 1H-[13C]-nuclear magnetic resonance spectroscopy measures of ketamine's effect on amino acid neurotransmitter metabolism, Biol. Psychiatry. 71 (2012) 1022–1025, https://doi.org/10.1016/j.biopsych.2011.11.006.
[44] S.M. Sears, S.J. Hewett, Influence of glutamate and GABA transport on brain excitatory/inhibitory balance, Exp. Biol. Med. 246 (2021) 1069–1083, https://doi.org/10.1177/1535370221989263.

[45] A.G. Evers, J.W. Murrough, D.S. Charney, S. Costi, Ketamine as a prophylactic resilience-enhancing agent, Front. Psychiatry. 13 (2022) 833259, https://doi.org/10.3389/fpsyt.2022.833259.
[46] I. Amelio, F. Cutruzzolá, A. Antonov, M. Agostini, G. Melino, Serine and glycine metabolism in cancer, Trends Biochem. Sci. 39 (2014) 191–198, https://doi.org/10.1016/j.tibs.2014.02.004.
[47] H. Ariyama, N. Kono, S. Matsuda, T. Inoue, H. Arai, Decrease in membrane phospholipid unsaturation induces unfolded protein response, J. Biol. Chem. 285 (2010) 22027–22035, https://doi.org/10.1074/jbc.M110.126870.
[48] D. Cavaleri, F. Bartoli, C.A. Capogrosso, et al., Blood concentrations of neopterin and biopterin in subjects with depression: A systematic review and meta-analysis, Prog. Neuropsychopharmacol. Biol. Psychiatry. 120 (2023) 110633, https://doi.org/10.1016/j.pnpbp.2022.110633.
[49] M. El Mansari, B.P. Guiard, O. Chernoloz, R. Ghanbari, N. Katz, P. Blier, Relevance of norepinephrine-dopamine interactions in the treatment of major depressive disorder, CNS Neurosci. Ther. 16 (2010) e1–e17, https://doi.org/10.1111/j.1755-5949.2010.00146.x.
[50] F. Bartoli, R.M. Cioni, D. Cavaleri, T. Callovini, C. Crocamo, B. Misiak, J.B. Savitz, G. Carrà, The association of kynurenine pathway metabolites with symptom severity and clinical features of bipolar disorder: An overview, Eur. Psychiatry. 65 (2022) e82.
[51] F. Bartoli, B. Misiak, T. Callovini, D. Cavaleri, R.M. Cioni, C. Crocamo, J.B. Savitz, G. Carrà, The kynurenine pathway in bipolar disorder: a meta-analysis on the peripheral blood levels of tryptophan and related metabolites, Mol. Psychiatry. 26 (2021) 3419–3429, https://doi.org/10.1038/s41380-020-00913-1.
[52] F. Bartoli, R.M. Cioni, T. Callovini, D. Cavaleri, C. Crocamo, G. Carrà, The kynurenine pathway in schizophrenia and other mental disorders: Insight from meta-analyses on the peripheral blood levels of tryptophan and related metabolites, Schizophr. Res. 232 (2021) 61–62, https://doi.org/10.1016/j.schres.2021.04.008.
[53] J.K.E. Veraart, S.Y. Smith-Apeldoorn, I.M. Bakker, et al., Pharmacodynamic Interactions Between Ketamine and Psychiatric Medications Used in the Treatment of Depression: A Systematic Review, Int. J. Neuropsychopharmacol. 24 (2021) 808–831, https://doi.org/10.1093/ijnp/pyab039.
[54] R.S. McIntyre, M. Alsuwaidan, B.T. Baune, et al., Treatment-resistant depression: definition, prevalence, detection, management, and investigational interventions, World Psychiatry. 22 (2023) 394–412, https://doi.org/10.1002/wps.21120.
[55] S. Chaki, M. Watanabe, mGlu2/3 receptor antagonists for depression: overview of underlying mechanisms and clinical development, Eur. Arch. Psychiatry Clin. Neurosci. 273 (2023) 1451–1462, https://doi.org/10.1007/s00406-023-01561-6.
[56] J.M. Witkin, K.P. Pandey, J.L. Smith, Clinical investigations of compounds targeting metabotropic glutamate receptors, Pharmacol. Biochem. Behav. 219 (2022) 173446, https://doi.org/10.1016/j.pbb.2022.173446.