Prebiotic Supplementation Modulates the Gut Microbiome for Improving Oral Antipsychotic Bioavailability

Kate Collins,[a] *Srinivas Kamath,*[a] *Tahlia R. Meola,*[a] *Anthony Wignall,*[a] *and Paul Joyce*[a] *

[a] UniSA Clinical & Health Sciences, University of South Australia, Adelaide, South Australia 5000, Australia

*Corresponding Author. Phone: +61 8 8302 1429; Email: paul.joyce@unisa.edu.au'; ORCID: 0000-0003-3619-7901

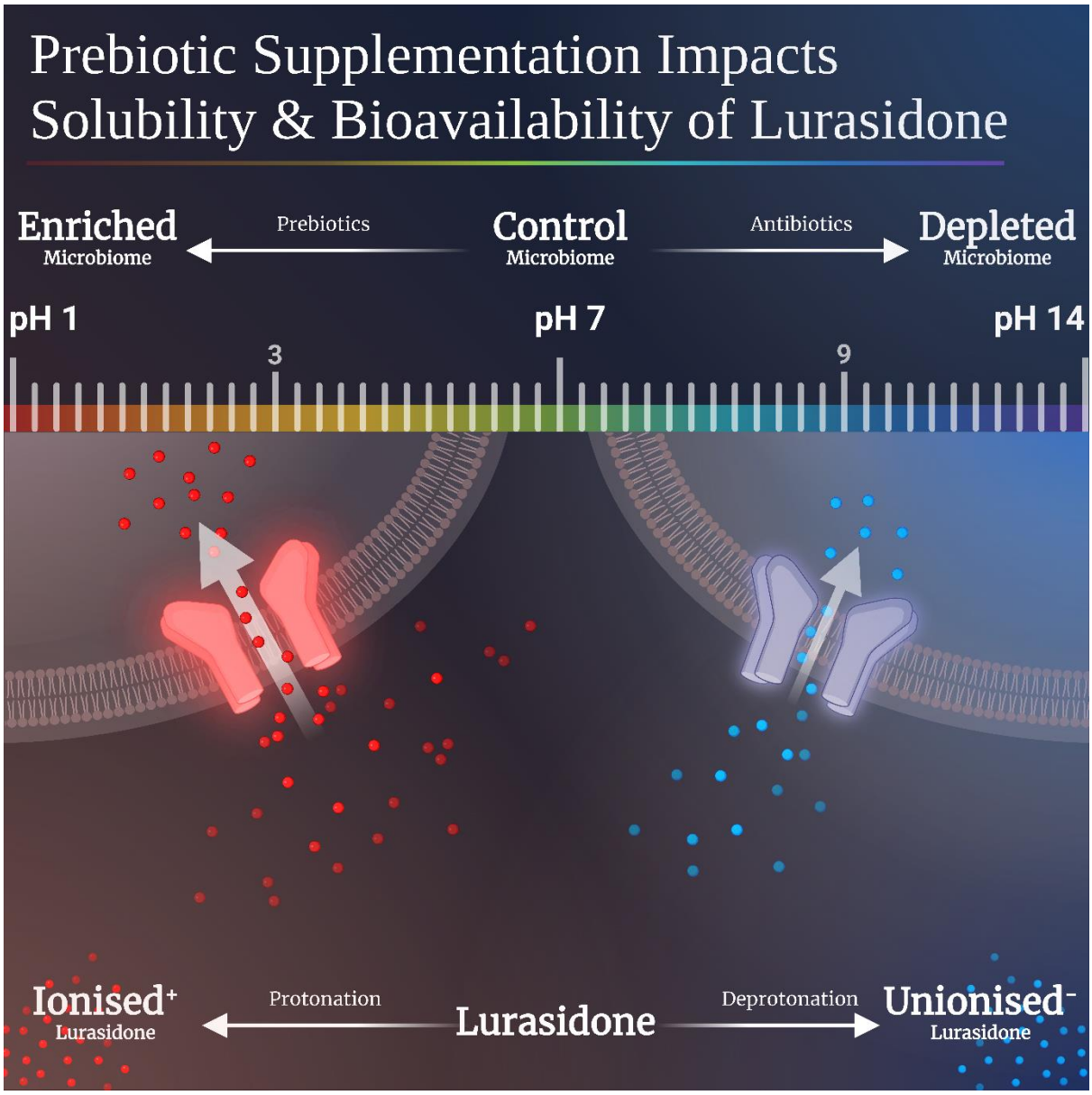
Abstract:

This study investigates the impact of gut microbiome composition on the bioavailability and biodistribution of lurasidone hydrochloride, an atypical antipsychotic used in treating mental health conditions. The research aims to uncover the relationship between gut metabolome, specifically short-chain fatty acids (SCFAs), and lurasidone absorption through modulation of intestinal pH. Male Sprague-Dawley rats underwent a 14-day microbiome depletion using antibiotics or prebiotics administered via drinking water, resulting in depleted, enriched, or normal (control) microbiomes. Following this treatment, lurasidone was orally administered to fasted rats, and blood samples were collected at specific intervals to correlate pharmacokinetic analysis with changes in microbiota and metabolome composition. Results revealed a significant 4.3-fold increase in lurasidone bioavailability following prebiotic administration, while antibiotic treatment decreased bioavailability. This effect is attributed to prebiotics increasing microbial diversity, which strongly correlates with SCFA production ($R^2 = 0.93$). The subsequent lowering of intestinal pH created a more suitable environment for lurasidone solubility and absorption. This novel study establishes a clear relationship between gut microbiome composition and lurasidone bioavailability, with prebiotic administration positively impacting both gut microbial diversity and drug absorption. Thus, these findings suggest that gut microbiome manipulation may present an innovative approach to improving therapeutic outcomes for antipsychotic medications, potentially addressing poor response rates in some patients. Furthermore, this research highlights the importance of considering gut microbiome composition, in particular the use of SCFAs as a biomarker to produce predictive models of drug pharmacokinetics, especially for pH-dependent soluble drugs. In the context of drug efficacy these gut microbiome-based predictive pharmacokinetic models will pave the path for personalised medicine approaches for mental health treatment.

Keywords:

Gut microbiome; Lurasidone; Bioavailability; Solubility; Antipsychotics; Short-chain fatty acids

Graphical Abstract:



1. Introduction:

Atypical antipsychotics pharmacotherapeutics are utilised for a broad spectrum of mental health conditions (such as major depressive disorder, anxiety, schizophrenia, and bipolar disorder) [1–3]. However, these medications are often prescribed without considering the potential negative effects, such as metabolic dysfunction and worsening mental health. Antipsychotics interact with the gut microbiome in a bidirectional way, which is frequently overlooked [1,4–6]. This interaction can lead to adverse effects, including metabolic disruptions like weight gain [4]. The bidirectional relationship involves two aspects: drugs and their excipients can disrupt the gut microbiome leading to adverse metabolic effects (*e.g.*, weight gain in the case of antipsychotics), and alternatively the gut microbiome can alter the pharmacokinetics of orally administered drugs (*e.g.*, absorption, biodistribution, metabolism, and excretion) [1,3,7,8].

A noteworthy example of a drug-gut interaction occurs with lurasidone, a benzothiazole derivative atypical antipsychotic that possesses pH-dependent solubility. This impact was previously explored by Cussotto et al. on similar atypical antipsychotics, olanzapine and risperidone [6]. Post microbiome depletion on adult male Sprague-Dawley rats (n: 6-7/group, weight: 0.200-0.250 kg, dosed: ampicillin 1g/L, vancomycin 500mg/L, and imipenem 250mg/L for 14 days in drinking water), olanzapine bioavailability increased 82%, whilst risperidone did not show any significant changes [6]. Cussotto et al. suggest pH changes and enzymatic degradation to be causative culprits however, they admit the discrepancy demonstrates that the impacts of the gut microbiome are not uniform [6]. Such mechanisms rationalise the real-world interindividual pharmacokinetic variation observed when taking antipsychotics and are corroborated by Minichino et al. [3].

Furthermore, short-chain fatty acids (SCFAs; acetate, propionate, and butyrate) are produced by microbial metabolism of indigestible dietary fibres which, as explored by Walker et al.,

influence intestinal pH [9]. This phenomenon in turn drives intestinal environments toward or away from the pKa of administered drugs [10]. Enrichment of the gut microbiome enhances SCFAS production, as several studies by Venkataraman et al., Deehan et al., McOrist et al. and Gibson et al. [11–14]. However, the implications of this for individual drugs are poorly understood.

This novel study aims to address this critical gap in our understanding of atypical antipsychotic pharmacokinetics by investigating the bidirectional relationship between microbiota and lurasidone. Unravelling these complex interactions and mechanisms underlying the poor solubilisation and bioavailability of lurasidone, may pave the way for more personalised and effective treatment strategies, potentially mitigating adverse effects and improving therapeutic outcomes for patients with mental health disorders. Furthermore, insights gained from this research could have far-reaching implications for drug development and administration across various classes of psychotropic medications, ultimately contributing to more informed and nuanced approaches in psychopharmacology.

2. Materials and methods

2.1 Animal Ethics & Husbandry

The *in vivo* study received approval from the Animal Ethics Committee at the University of South Australia (approval #U24-21), adhering to the NIH's Principles of Laboratory Animal Care (NIH publication #85-23), revised in 1985) and the Australian code for the care and use of animals for scientific purposes (8th edition 2013, revised 2021), and was conducted and reported following ARRIVE guidelines. The study was conducted on 8-week-old male Sprague-Dawley rats sourced from Ozgene (Canning Vale, Australia) with a mean starting weight of 0.270-0.330 kg. Sprague-Dawley rats were utilised in this study because 1) their gut microbiome closely mimics the human gut microbiome [15,16], 2) they are widely utilised in pharmacomicrobiomics studies [17,18] and have been used previously to investigate the impact of

olanzapine on the gut microbiome [4] and 3) their docile nature allows for ease-of-handling for daily dosing via oral gavage [19]. Rats were housed in groups of 2 in a temperature-, humidity-, pressure-controlled animal facility with a 12h/12h light/dark cycle with access to standard rodent chow and water. Only healthy rats were included in the study, and those showing signs of illness or abnormal behaviour during the acclimation period were excluded. Randomisation and blinding were implemented during the allocation of animals to treatment groups and throughout the data collection process. The researchers administering treatments, as well as those assessing outcomes, were blinded to the group allocations to prevent bias.

2.2 In vivo study design

The oral bioavailability of lurasidone (Hangzhou Dayangchem Co. Ltd., Hangzhou City, China) was investigated in three randomly assigned treatment groups ($n = 5$). The sample size for each treatment group ($n = 5$) was based on Power calculations for anticipated changes in the lurasidone area under the curve, using a power level of ≥ 0.8 and a significance level of 0.05. A single-gender was used for the pharmacokinetics study to minimise gender-based variation commonly observed in oral pharmacokinetics investigations. Treatment groups were as follows: 1) control group where rats were provided *ad libitum* access to standard drinking water; 2) enriched microbiome group where rats were provided *ad libitum* access to drinking water with inulin (100 mg/L) dissolved; and, 3) depleted microbiome group where rats were provided *ad libitum* access to drinking water with vancomycin (500 mg/L), ampicillin (1 g/L), and imipenem (250 mg/L) dissolved. Following 14 days of pre-treatment with prebiotics (*i.e.,* inulin, inulin from dahlia tubers (with a degree of polymerisation of 35) were purchased from Merck [Bayswater, Australia]) or antibiotics (ampicillin and vancomycin were purchased from Merck [Bayswater, Australia]) to manipulate the gut microbiome, each treatment group was administered a single dose of lurasidone (7.5 mg/mL in PBS) via oral gavage. Blood samples

were collected over various time points, (0, 0.33, 0.66, 1, 1.5, 2, 4, 6, 8, 24h) for pharmacokinetic analysis. Caecal samples were collected for gut microbiome analysis. After the final samples were collected, rats were anesthetised using 5% isoflurane, followed by cardiac puncture and cervical dislocation.

2.3 Gut microbiota analysis

Following treatment and a single dose of lurasidone animal caecal contents were collected and stored in a -80°C freezer for future analysis. Samples were sent to the Australian Genomics Research Facility (Brisbane, Australia) for DNA extraction, PCR amplification, and 16S rRNA sequencing. Using the Quantitative Insights to Microbiology Ecology (QIIME 1.8and the Silvia reference database the 16S rRNA sequences were processed for the V3-V4 hypervariable regions, with raw reads clustered into operational taxonomic units (OTUs). OTUs were assigned taxonomy using the QIAGEN Microbial Insights – Prokaryotic Taxonomy Database (QMI-PTDB) within the QIAGEN CLC Genomics Workbench version 23.0.4 (Hilden, Germany). Alpha diversity was measured via Shannon's Index at the genus level, and beta diversity analyses were conducted using Principal Coordinate Analyses (PCoAs) based on Bray-Curtis' dissimilarity metrics. Statistical significance was evaluated through Permutational Multivariate ANOVA (PERMANOVA) on the beta diversity plots.

2.4 Short-chain fatty acid analysis

SCFA concentration in caecal contents was analysed using a modified gas chromatography-mass spectrometry (GCMS) protocol (Moreau et al, 2003), modified for the GC–MS (7890A GC System and 5975C inert XI EI/CI MSD with an EI inert 350 source) and MassHunter Workstation Software (MassHunter, Agilent Technologies). The protocol details have been previously published [9].

2.5 Intestinal pH analysis

Rat intestinal media was analysed to investigate the effect of the gut microbiome on intestinal pH. A Hanna instruments Bluetooth pH electrode (HI10832) was used to measure intestinal pH. The electrode was placed in the intestinal media and the measurement was recorded.

2.6 Lurasidone drug solubility

Lurasidone drug solubilisation was analysed by adjusting the pH of the simulated intestinal buffer solution and adding lurasidone in excess. pH levels 6.25, 6.75, and 7.5 were of interest due to being common within rat intestinal media. The different pH levels were made using intestinal buffer and hydrochloric acid to achieve the pH level desired. The Hanna instruments Bluetooth pH electrode (HI10832) was used to verify the pH level. Lurasidone was added to the pH buffers in excess, above its solubility, with 1 mg of lurasidone added to 1 mL of each different pH solution. Investigations were performed in triplicate. Lurasidone and pH buffer solution were left on a moving plate at 37 degrees for 2 days covered in aluminium. Samples were centrifuged at max speed for 20 minutes to allow lurasidone precipitate to be separated. The supernatant was collected and analysed using liquid chromatography-mass spectrometry (LCMS). The protocol details have been previously published [10].

2.7 Pharmacokinetic analysis

Blood samples were collected from the saphenous vein and centrifuged to collect the plasma. For LCMS analysis the plasma was diluted with acetonitrile (5:9 ratio) and spiked using an internal standard (ziprasidone; 10µl). Pharmacokinetic analyses were performed using Phoenix® WinNonlin® Version 8.3 (Pharsight®, a Certara™ company), using non-compartmental methods (Model 200–202, Extravascular Input) and a linear-trapezoidal approach.

2.8 Statistical Analysis

GraphPad Prism Version 8.0 (GraphPad Software Inc., California) was used for all statistical analyses (excluding 16S sequencing analyses) of experimental data. An unpaired t-test and one-way ANOVA were used to determine statistically significant differences, followed by Tukey's post-test for multiple comparisons. Values are expressed as the mean ± standard deviation (SD), with statistical significance set at $p < 0.05$. In the figures, statistical significance is denoted by * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, and **** $p < 0.00005$.

3. Results

3.1 Gut microbial enrichment via prebiotic supplementation correlates with metabolome enrichment through increased SCFA concentrations in the caecum.

An overview of the dosing regimen and study design to investigate the impact of the gut microbiome on lurasidone bioavailability is presented in **Fig. 1A.** The 14-day pre-treatment approach, whereby treatment groups were administered 1) no pre-treatment (*i.e.*, control), 2) antibiotics, or 3) prebiotics *ad libitum* in drinking water. Analysis of microbial abundance at the family taxonomic level from the caecal contents from each animal, illustrated in **Fig. 1B**, with grouped data highlighted in **Fig. 1C**, indicates that the pre-treatment led to treatment groups with a 1) control microbiome, 2) depleted microbiome, and 3) enriched microbiome. Microbial abundance increased following prebiotic pre-treatment, in particular members of the *Lachnospiraceae* and *Bacteroidacae* families. In contrast, Enterobacteriaceae show predominance in the antibiotic-treated group. Observed changes in total operation taxonomic units (OTUs) and alpha diversity (measured by Shannon's index) are shown in Fig. 1D and Fig. 1E for the three groups. The prebiotic treatment group exhibits an enriched microbiome via an increase in OTUs (+50 OTUs) and Shannon's index (+1 increase in Shannon's index) compared to the control group. Contrary to this, the antibiotic treatment group displays a significant

decrease in OTUs (-50 OTUs) and Shannon's index (-1 decrease), when compared to the control group. PERMANOVA of principal component analysis (PCoA) reveals statistically significant differences in beta diversity between the three treatment groups (Fig 1F), further highlighting that three distinct microbial populations were present for the respective treatment groups.

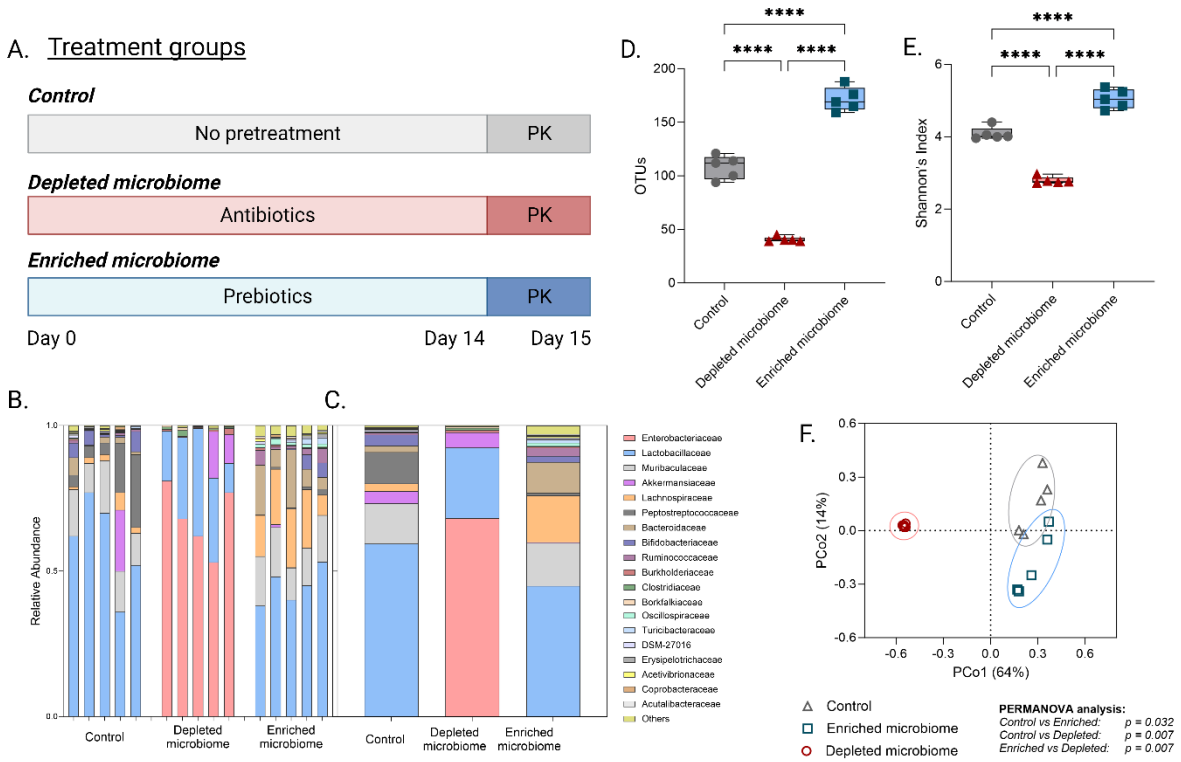


Figure 1. ***Microbial pretreatment resulted in three distinct microbiome compositions between the treatment groups***. *[**A**] A schematic representation of the treatment protocols for each group. Relative abundance of gut microbiota families post-treatment for individual animals (Panel [**B**]) and grouped averages (Panel [**C**]). Changes in OTUs (Panel [**D**]) and Shannon's Index (Panel [**E**]) reveal statistically significant changes in microbial diversity across the three treatment groups. Principal component analysis demonstrates statistically significant shifts in microbiota composition across the three treatment groups (Panel [**F**]). Statistical significance*

was assessed using unpaired t-test and one-way ANOVA for α-diversity and PERMANOVA for β-diversity. The asterisk (****) denotes statistically significant results ($p < 0.0001$).

The concentration of key SCFAs was quantified within caecal contents. A significant difference ($p = 0.0004$) in acetic acid concentration was observed between the enriched microbiome and depleted microbiome groups (**Fig. 2A**). Similarly, propionic acid concentrations are reduced ($p = 0.0296$) in microbiome-depleted groups compared to the control group (**Fig. 2B**). No significant difference in butyric acid concentration is noted between control and enriched microbiome groups, with a similar concentration range (10-20 μmol g^{-1}), however, depleted microbiome group showcased a significant decrease (5 μmol g^{-1}), as depicted in **Fig. 2C**. Total SCFA concentrations vary between all groups as depicted in **Fig. 2D**. The enriched microbiome demonstrates the highest concentration (200-300μmol g^{-1}), while the depleted microbiome has the lowest total SCFA concentration (< 50 μmol g^{-1}). A strong correlation between total SCFA (μmol g^{-1}) and Shannon's index ($R^2 = 0.93$) is displayed in **Fig. 2E**, suggesting microbial diversity increases total SCFA concentration. Similarly, a correlation ($R^2 = 0.83$) can be noted between total SCFA (μmol g^{-1}) and OTUs (**Fig. 2F**).

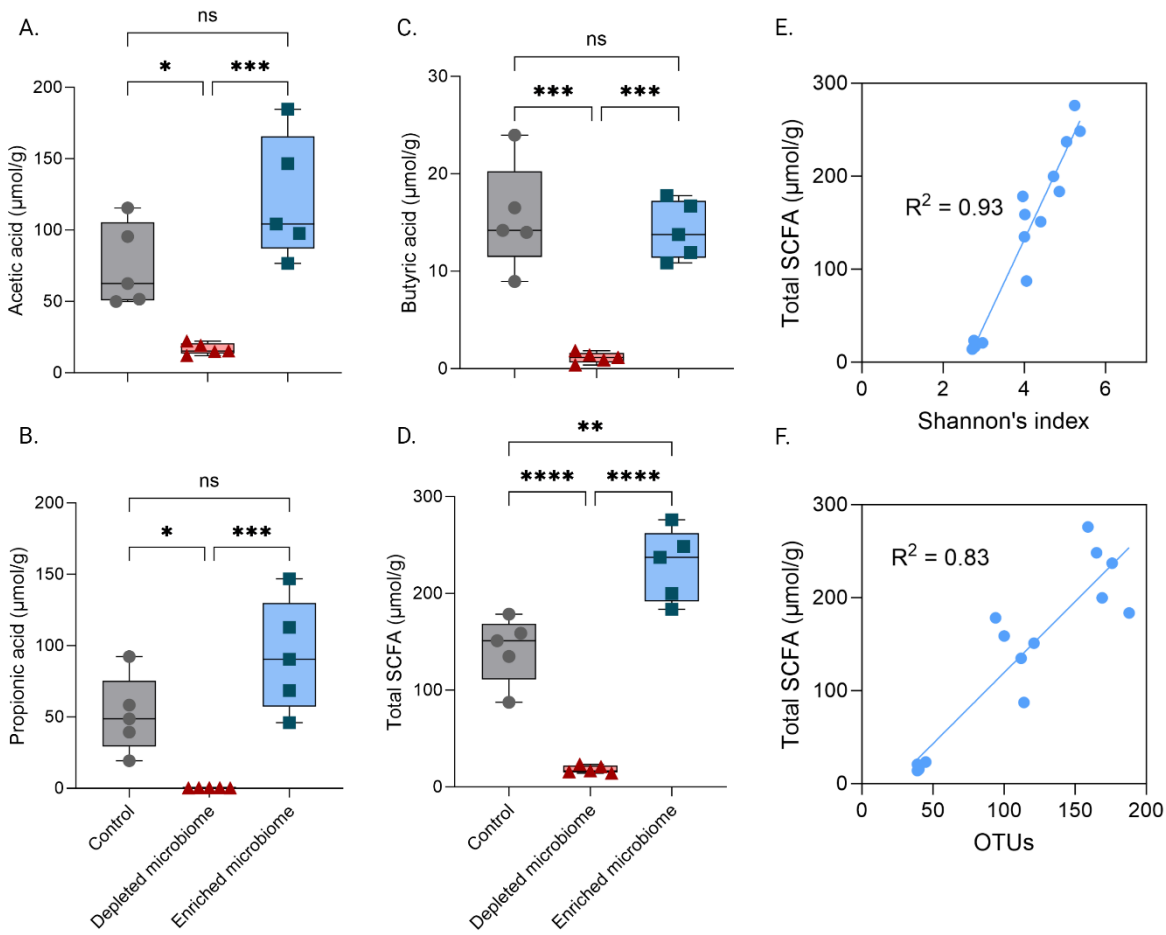
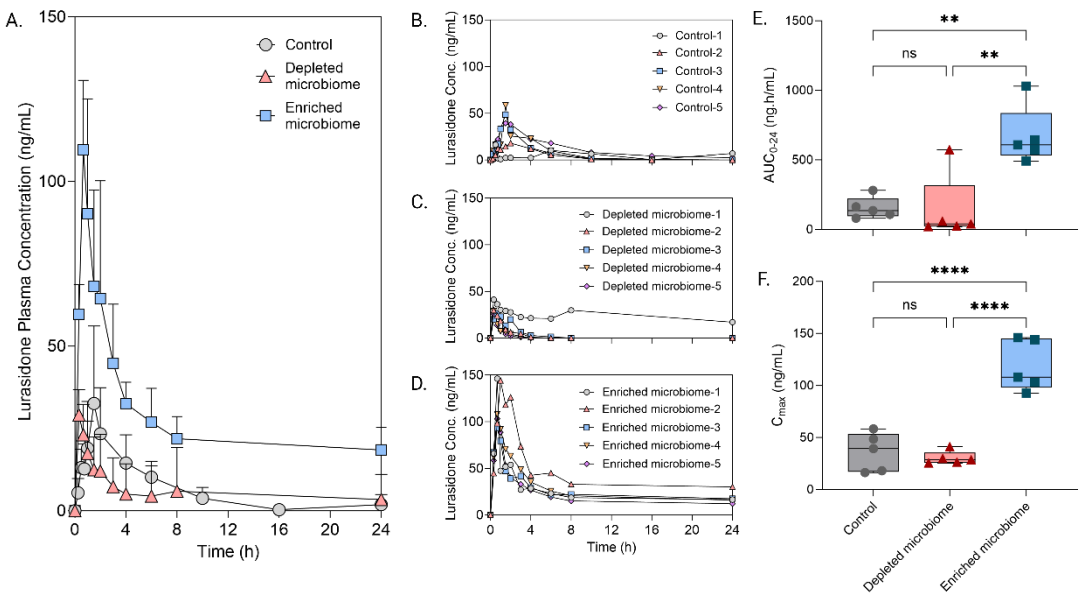


Figure 2. ***Enriched microbiota diversity correlates directly with an increase in SCFA concentrations in rats.*** *[**A**] Acetic acid concentration across control, depleted, and enriched microbiome groups. [**B**] Propionic acid concentration across the three groups. [**C**] Butyric acid concentration across the three groups. [**D**] Total SCFA concentration across the three groups, demonstrating statistical significance across all three groups investigated. Total SCFA concentration directly correlates with alpha diversity, as demonstrated through strong correlations with Shannon's index, showing strong correlation (R^2 = 0.93) (Panel [**E**]) and OTUs (R^2 = 0.83) (Panel [**F**]). Values are presented as the mean ± standard deviation (SD). Statistical significance was assessed using an unpaired t-test and one-way ANOVA. The asterisk (*), (**), (***) or (****) denote statistically significant results ($p < 0.05$), ($p < 0.01$), ($p < 0.001$) or ($p < 0.0001$) respectively.*

226 *3.2 Increased lurasidone plasma concentration in enriched microbiome*

227 The pharmacokinetic profiles for each group after a single oral dose of lurasidone (20 mg/kg)
228 to male Sprague Dawley rats are displayed in **Fig. 3A** with interindividual pharmacokinetic
229 variability highlighted in **Fig. 3B-D.** Noteworthy is the outlier present in the depleted
230 microbiome group (**Fig. 3C**) which showcases uncharacteristically prolonged lurasidone
231 absorption/ delayed clearance. When excluded from consideration, the mean lurasidone
232 bioavailability is diminished. The enriched microbiome group demonstrates a 4.3-fold increase
233 in lurasidone bioavailability over the 24 hours in comparison to the control, based on mean
234 area-under-the-curve ($AUC_{0\text{-}24}$) values, presented in **Fig. 3E**. Further, C_{max} values increased by
235 3.3-fold and 3.9-fold for the enriched microbiome group compared to the control and depleted
236 microbiome group, respectively (**Fig. 3F**). The degree of drug absorption between the control
237 and depleted microbiome groups were statistically equivalent, but significant differences were
238 observed in T_{max} values. The pharmacokinetic results are summarised in Table 1.



239 **Figure 3.** ***Microbiome enrichment via prebiotic supplementation increases lurasidone***
240 ***bioavailability****. [A] Pharmacokinetic profiles for the oral administration of lurasidone (20*
241 *mg/kg) across the three groups (n = 5) revealed an increase in oral bioavailability for the*

*enriched microbiome group. Panels [**B-D**] present the pharmacokinetic analysis of each rat within the three groups. [**E**] Showcasing area-under-the-curve across the groups with statistical significance observed between enriched microbiome and depleted microbiome groups, and enriched microbiome and control groups respectively. [**F**] Maximum lurasidone plasma concentration observed across the three groups trialled. Values are presented as the mean ± standard deviation (SD). Statistical significance was assessed using an unpaired t-test and one-way ANOVA. The asterisk (**) or (****) denote statistically significant results (p< 0.01) or (p < 0.0001) respectively.*

Table 1: Pharmacokinetic parameters following oral administration of lurasidone (20 mg/kg) to male Sprague Dawley rats in the fasted state following a 14-day pre-treatment with antibiotics and prebiotics to deplete and enrich the gut microbiome, respectively. Values represent the mean ± SD, *n* = 5.

Pharmacokinetic parameter	Control	Depleted microbiome	Enriched microbiome
T_{max} (h)	1.40 ± 0.548	0.39 ± 0.15	0.736 ± 0.148
C_{max} (ng/mL)	36.2 ± 18.6	30.3 ± 6.30	119 ± 24.6
AUC_{0-24} (ng.h/mL)	154 ± 77.3	144 ± 241	669 ± 207
$T_{1/2}$ (h)	3.07 ± 2.13	0.863 ± 0.20	Not determined

3.3 SCFAs lower intestinal pH and promote lurasidone solubility

The impact of microbiome compositional changes on luminal pH was investigated, with **Fig. 4A**. demonstrating significant alteration to intestinal pH accompanied by gut microbiome changes. An enriched microbiome results in a lower pH (6.0-6.5), and a depleted microbiome shows a higher pH (7.0-8.0), compared to the control group exhibiting a pH range of 6.5-7.0. Strong negative correlations existed between intestinal pH and Total SCFA (**Fig. 4B**) and Shannon's Index (**Fig. 4C**), demonstrating that the intestinal pH decreases as a function of increasing SCFA concentrations. Lower pH levels in the enriched microbiota groups also demonstrated 2-fold increases in lurasidone solubility compared to control groups (**Fig. 4D**).

Fig. 4E and **4F** demonstrate correlations between area-under-the-curve and total SCFA (R^2 of 0.72) and Shannon's index (R^2 of 0.71) respectively.

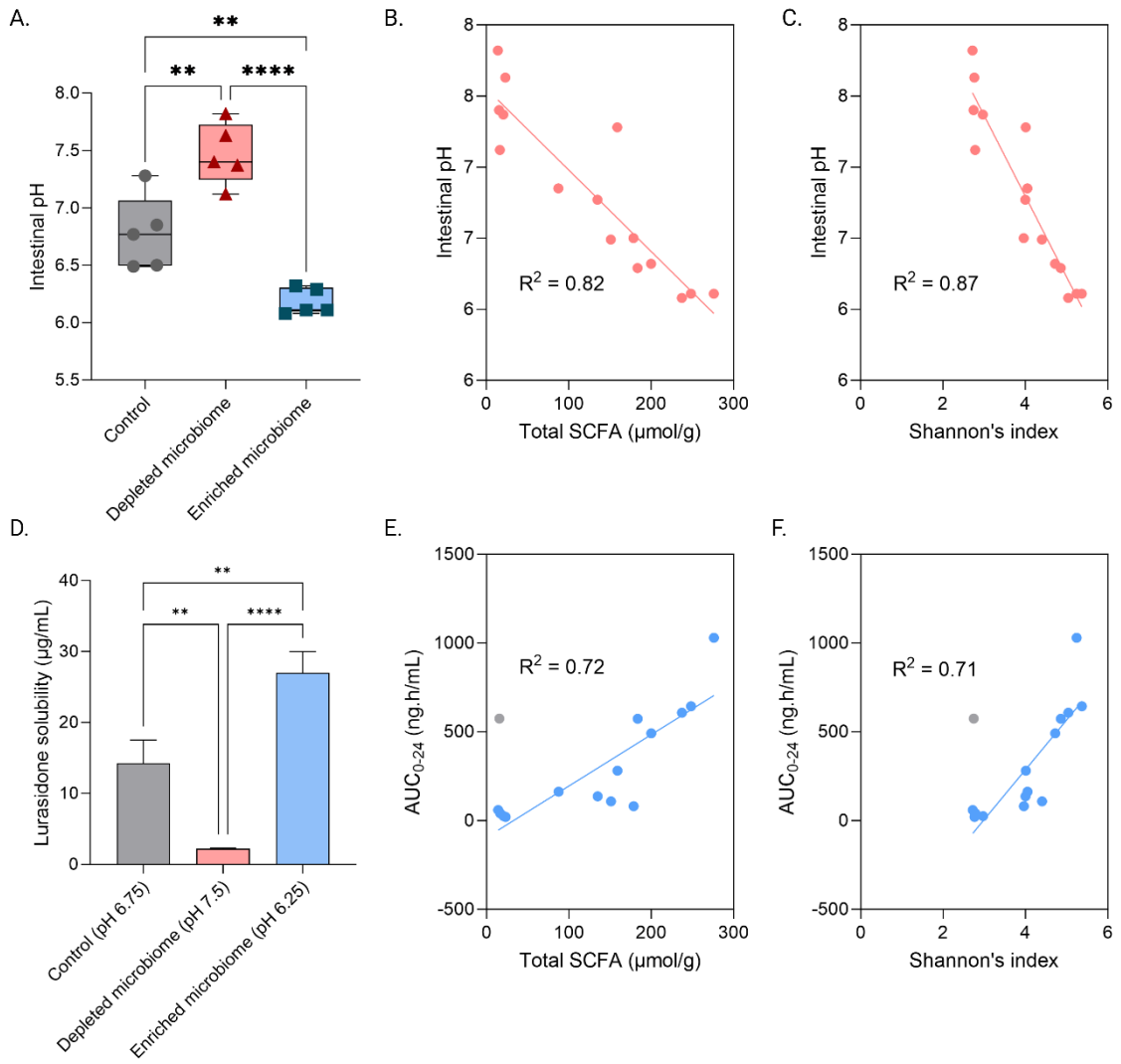


Figure 4. ***An enriched microbiome demonstrates increased lurasidone solubility due to a decreased intestinal pH.*** *[**A**] Intestinal pH levels across control, depleted microbiome, and enriched microbiome. The enriched microbiome displays the lowest pH level and is statistically significant. [**B**] Illustrating the relationship between total SCFAs and intestinal pH there is a strong correlation (R^2 = 0.82). [**C**] Exhibiting Shannon's index against intestinal pH, with a strong correlation (R^2 = 0.87). [**D**] Lurasidone solubility across the three groups. An enriched microbiome demonstrates the highest lurasidone solubility and statistical significance when compared with the depleted microbiome. [**E**] and [**F**] Highlighting area-under-the-curve*

against total SCFA and Shannon's index. Both show strong correlation (R^2 = 0.71 and 0.72) Values are presented as the mean ± standard deviation (SD). Statistical significance was assessed using an unpaired t-test and one-way ANOVA. The asterisk (**) or (****) denote statistically significant results (p< 0.01) or (p < 0.0001) respectively.

4. Discussion

Pharmacomicrobiomics explores the bidirectional relationships between drugs and the gut microbiome and is of particular importance when prescribing antipsychotic medication [1,20–22]. Whilst the impact of psychotropics on the gut microbiome is gaining traction with comprehensive reviews from Kamath et al. and Minichino et al., the reciprocal relationships are less explored [1,3].

To investigate the pharmacomicrobiomics of lurasidone, an *in vivo* animal model of Sprague-Dawley rats was utilised with an antibiotic, control and prebiotic group (as per **Fig. 1A**) with doses interpreted from prior work by Huang et al. and Davey et al. [4,23]. Antibiotic supplementation decreased several commensal bacterial families such as *Lachnospiraceae* and *Bacteroidacae* both of which are also vital for SCFA production as per prior work by Fusco et al. [24]. *Lachnospiraceae, in* particular, is suggested by Vacca et al. to possess several members (such as *Roseburia* and *Eubacterium rectale*) which ferment starch and simple sugars into butyrate (via the butyryl-CoA pathway), formate and lactate [25]. Similarly, *Bacteroidacae* harbour polysaccharide utilisation loci responsible for saccharification of complex carbohydrates, which result in SCFA production as suggested by Zhang et al. [26]. Enrichment of the gut microbiome increased both *Lachnospiraceae* and *Bacteroidacae* relative abundance alongside holistic improvements in OTUs (**Fig. 1D**) and increases in β-diversity (**Fig. 1F**) both of which align with previous studies by Carlson et al. and Tian et al. [27,28]. Such increases in the relative abundance of the aforementioned SCFA-producing bacteria and overall OTUs led to

increases in SCFA concentrations (**Fig. 2D**) with strong correlations being noted between SCFA concentration and Shannon's index and OTUs (**Fig. 2E**: R^2 0.93, **Fig. 2F**: R^2 0.83). Such results are echoed by Gurry et al. with similar correlations between functional heterogeneity in gut microbiome and SCFA production [29].

The increases in SCFA bear importance in exploring pharmacomicrobiomics through their influence on luminal pH. SCFAs are acidic (propionate: pH 2.4, butyrate: pH 5.5, acetate: pH 4.6), which Liu et al. suggest can decrease luminal pH through the release of H^+ ions [30]. Specifically, the acidity of SCFAs arises from the presence of a carboxyl group (-COOH), which can donate a proton (H^+) to the solution, forming a carboxylate anion ($R\text{-}COO^-$) stabilised by resonance as suggested by Parada Venegas et al. [31]. Acetic acid (CH_3COOH) releases a proton to form acetate (CH_3COO^-), propionic acid (CH_3CH_2COOH) forms propionate ($CH_3CH_2COO^-$), and butyric acid ($CH_3CH_2CH_2COOH$) forms butyrate ($CH_3CH_2CH_2COO^-$). The inductive effect of the alkyl chains (methyl, ethyl and propyl) also slightly influences their acidity, with longer chains being less acidic due to electron-donating effects. Sun et al. explored the physiological role of SCFAs, suggesting this luminal pH reduction to be integral in inhibiting pathogenic invasion and colonisation [32]. However, in the context of pharmacomicrobiomics, the acidic environment can drive drugs toward or further from their pKa, influencing drug absorption, metabolism, and efficacy as noted by Panebianco et al. [20].

Lurasidone, (chemical formula: C28H36N4O2S) contains a benzothiazole derivative with a piperazine ring (providing basicity and contributing to pKa), a sulphonamide group (neutral but capable of influencing hydrogen bonding capacity), and multiple aromatic rings (impacting lipophilicity and drug-receptor interactions) which result in a pKa of 7.6 [33]. Below the pKa, the piperazine nitrogen is protonated, resulting in a positively charged species (Lurasidone-NH_3^+)

which possesses greater aqueous solubility and hydrophilicity, through the formation of ion-dipole interactions between the positively charged amine and water molecules [34,35]. This results in the maximum aqueous solubility of 0.349 mg/mL in pH 3.5 buffer [33].

Whilst the specifics of lurasidone absorption and active transporter receptors is poorly understood (possibly due to its recency in development), increased solubility is generally accepted as being favourable towards increasing absorption and bioavailability as covered in a comprehensive review by Kumari et al. [36]. Alternative atypical antipsychotics also possess similar pH-dependent solubility characteristics, such as olanzapine (protonation of amine group) and risperidone (protonation of the piperidine nitrogen) [37]. However, unlike alternatives, lurasidone is hampered by poor bioavailability (9-19%) with a 2-fold positive food effect owing to its poor aqueous solubility [38]. Such findings may explain why lurasidone prescription rates are considerably lower than alternatives such as olanzapine (*e.g.,* 111,911 prescriptions for lurasidone in Australia compared to 719,915 for olanzapine) [39].

In the context of SCFA luminal accumulation, the lowered pH ensures lurasidone remains in its ionised form, thereby enhancing aqueous solubility [40]. Enriched microbiota groups correlated with increased OTUs (**Fig. 2D)** and subsequently increased SCFA production (**Fig. 2F**). Ultimately, these findings correlated strongly (**Fig. 4B:** $R^2 = 0.82$ and **Fig. 4C** $R^2 = 0.87$) with intestinal pH, suggesting enriched microbiomes presented decreased luminal pH as corroborated by Walker et al. [9]. The aforementioned pH-dependent solubility of lurasidone benefits from this SCFA-induced decrease in pH and thus the gut microbiome emerges as a key mediator between SCFAs, luminal pH and subsequent drug solubility and bioavailability. Such assertions are corroborated by **Fig. 4E** and **F**, with both exhibiting strong correlations between the gut microbiome, SCFAs and subsequent impacts on lurasidone $AUC_{0\text{-}24}$ (R^2 0.72 and 0.71

respectively). Whilst not previously explored for lurasidone, similar relationships are summarised in a comprehensive review by Kamath et al. [1].

By extension, the correlations between SCFA, pH and subsequent solubilisation, absorption and bioavailability of lurasidone illustrate a future wherein predictive models may use SCFAs as biomarkers for managing personalised therapy. Currently, SCFAs are tested as putative biomarkers for colorectal and non-small cell lung cancers [41]. However, these scenarios are limited in scope, restricted to merely suggesting gut inflammation characteristics and immune activation [42]. Emerging work by Zhao et al. and Tsunoda et al. suggests the gut microbiome composition as a whole to be capable of predicting drug pharmacokinetics [43,44]. A case example is demonstrated by Duan et al. which showcased discernible differences in the gut microbiome of escitalopram responders and non-responders (C57BL/6 male mice model, n: 7/group, mean age: 4-6 weeks) [45]. Furthermore, the gut microbiome has shown capacity to modulate hepatic cytochrome P450 (CYP450) characteristics as explored by Wang et al. and Bai et al. [46,47]. In the context of lurasidone, it presents another avenue of pharmacokinetic modulation as lurasidone is extensively metabolised (thought oxidative N-dealkylation between the piperazine and cyclohexane rings) by CYP450, forming lurasidone metabolites 14326, 14283 (both active) and 20219, 20220 (inactive) [48]. By harnessing the gut-liver axis (facilitated by intestinal permeability), the gut microbiome is capable of altering CYP450 levels and thereby lurasidone pharmacokinetics [49,50]. As such, identification of specific gut biomarkers (such as SCFAs metabolites) together with machine-learning based predictive models may provide personalised pharmacotherapy to mental health patients as explored in reviews by Keutzer et al. [51].

The insights derived from this study are valuable in broadening our understanding of pharmacomicrobiomics, in particular of poorly understood lurasidone and are summarised in **Fig 5**.

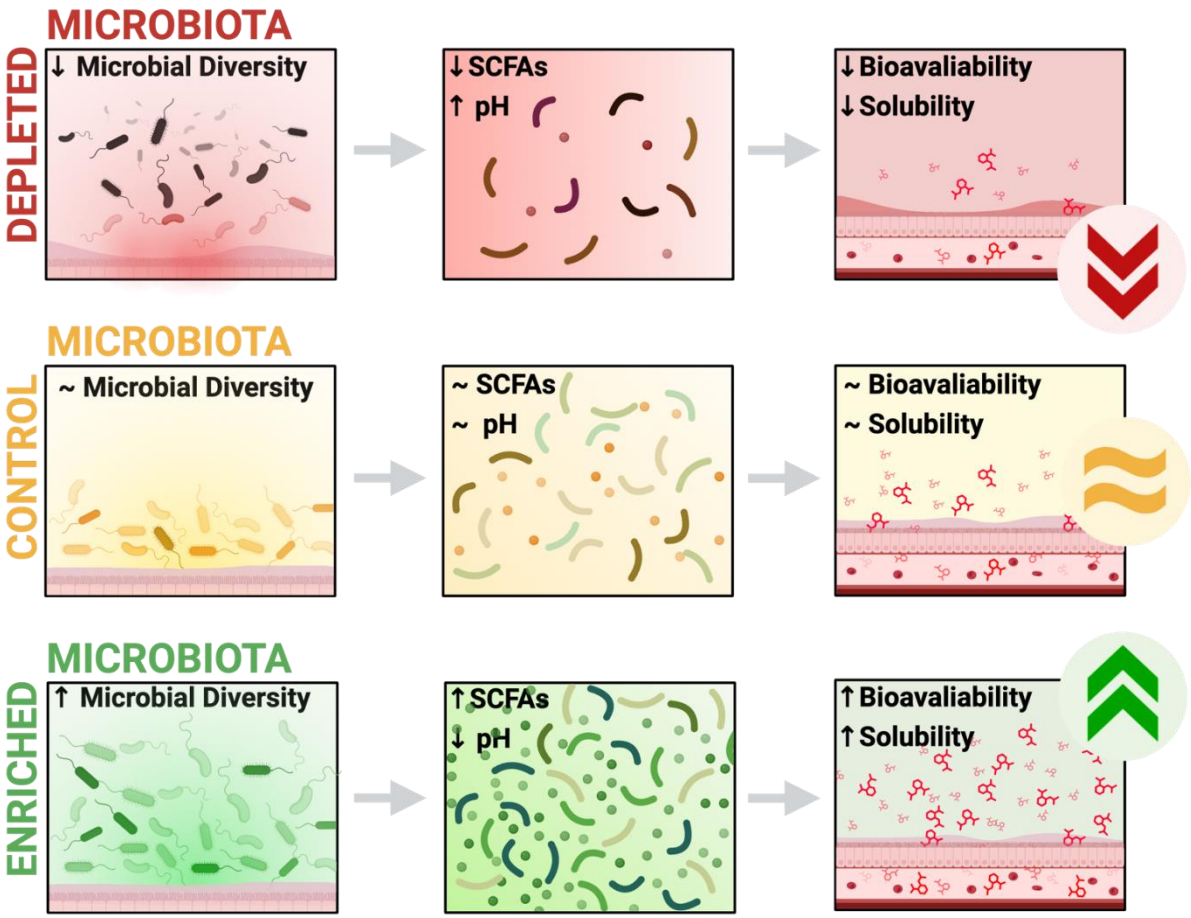


Figure 5. ***Enriched microbiome increases bioavailability and solubility, in contrast the depleted microbiome decreases bioavailability and solubility.*** *Three treatment groups and their subsequent effects on the gut microbiome and drug bioavailability and solubility.*

Given the role of pH and the gut microbiome in modulating lurasidone solubility and absorption, it may necessitate re-evaluation of treatment regimens for schizophrenia to accommodate gut microbiome interventions to maximise pharmacotherapy efficacy. Recent evidence that lurasidone presents 'gut neutral' characteristics and imparts generally favourable impacts on the gut microbiome, thus it may serve as a better-suited therapeutic option for

metabolic sensitive patients [5]. Furthermore, it is hoped that the exploration of lurasidone's pH-dependent solubility spur strategies to maximise solubility, absorption and bioavailability, for example as achieved by Meola et al. through the formulation of a lipid-based nanostructure and porous silica nanostructure which enhanced bioavailability of lurasidone by 8.8-fold. [52]. Similarly, Gamboa-Arancibia et al. formed a lurasidone-hydroxypropyl-β-cyclodextrin (obtained through the hydroxylation of β-cyclodextrin) and observed increases in solubility (up to 20 mg/mL) attributable to the presence of the hydroxyl substituent in this cyclodextrin [53]. Thus, it is expected that the current findings will guide future research in optimising therapy and potentially lead to increased prescription and clinical use of lurasidone in standard therapy for treating schizophrenia and bipolar disorder.

5. Conclusion and Prospects:

This study on lurasidone's pharmacomicrobiomics reveals the intricate relationships between the gut microbiome, SCFA production, luminal pH, and drug bioavailability. The critical role of commensal bacteria, particularly *Lachnospiraceae* and *Bacteroidaceae*, in modulating the gut environment highlights the importance of considering microbiome dynamics in antipsychotic treatment strategies. Lurasidone's pH-dependent solubility and 'gut neutral' characteristics position it as a promising option for metabolically sensitive patients, despite its poor bioavailability. Future research should focus on validating these findings in human studies, interpreting specific mechanisms of lurasidone absorption, and developing novel drug delivery systems to enhance bioavailability. The success of lipid-based nanostructures and cyclodextrin complexes in improving solubility suggests promising avenues for formulation development. These findings underscore the need to re-evaluate treatment regimens for schizophrenia and bipolar disorder, potentially incorporating additional gut microbiome interventions to maximise pharmacotherapy efficacy. Additionally, exploring personalised medicine approaches based on individual gut microbiome profiles and investigating the broader

implications for other pH-sensitive drugs could lead to significant advancements in drug development and administration.

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