Aging and Disease
www.aginganddisease.org
Early access date: May 4, 2026
http://dx.doi.org/10.14336/AD.2026.0375

Review

The Copper-Gut-Brain Axis: A Triple Inflammatory Pathway Driving Neuroinflammation in Alzheimer's Disease

Ashwin Ambi[1*]

[1]Enzo Life Sciences, Inc., Farmingdale, NY 11735, United States

[Received March 30, 2026; Revised May 1, 2026; Accepted May 2, 2026]

ABSTRACT: Serum copper increases progressively with normal aging, yet its downstream consequences for the gut microbiome and neuroinflammation remain unexplored. Gut microbiota dysbiosis and elevated lipopolysaccharide levels are established features of Alzheimer's disease, and growing evidence indicates that this dysbiosis drives neuroinflammatory disease progression. Yet the upstream trigger initiating this dysbiosis remains unknown. We propose that age-related copper dyshomeostasis serves as this missing trigger. The redox-active copper content of ceruloplasmin increases across the adult lifespan, and copper is selectively toxic to anaerobic bacteria, preferentially affecting butyrate-producing genera including Faecalibacterium, Roseburia, and Coprococcus while sparing copper-resistant species. This selective toxicity is supported by animal studies demonstrating copper-induced elimination of butyrate producers with reversible gut barrier damage and by Wilson's disease cohorts showing consistent depletion of butyrate-producing genera due to elevated copper levels. The resulting dysbiosis creates a triple inflammatory pathway: butyrate loss compromises gut barrier integrity and removes histone deacetylase-mediated suppression of neuroinflammation; the increase of Gram-negative bacteria elevates lipopolysaccharide translocation through the compromised barrier; and impaired blood-brain barrier integrity reduces amyloid-β clearance. These three insults trigger microglial activation through NF-κB signaling, creating a 'triple hit' on a single transcription factor that may explain the magnitude of neuroinflammatory effects observed in Alzheimer's disease. This mechanism explains the increased acetate/butyrate ratio recently identified as a biomarker distinguishing Alzheimer's-related from non-Alzheimer's cognitive impairment (AUC 0.951), since copper disrupts microbial metabolic cross-feeding networks that convert acetate to butyrate. We present specific, falsifiable predictions that can be tested in human cohorts and propose copper as a novel upstream therapeutic target for Alzheimer's disease prevention.

Keywords: aging, copper dyshomeostasis, gut-brain axis, Alzheimer's disease, butyrate, neuroinflammation, lipopolysaccharide, NF-κB, gut dysbiosis, blood-brain barrier

Introduction

The connection between the brain and the gut has emerged as a critical factor of the major pathways in the pathogenesis of neurodegenerative diseases due to altered microbiota [1]. The effect of this bidirectional communication has been evidenced in patients with Alzheimer's disease (AD) characterized by altered gut microbiome composition. Studies found decreased Firmicutes and increased Bacteroidetes in AD patients [2], along with elevated peripheral inflammation markers associated with pro-inflammatory gut bacteria [3]. The gut-brain axis works through neural pathways (particularly the vagus nerve), immune signaling, and metabolic routes [1, 4]. Genetic studies identified overlap between host genetic factors influencing gut microbiome composition and AD risk [5].

***Correspondence should be addressed to:** Dr. Ashwin Ambi, Enzo Life Sciences, Inc., Farmingdale, NY 11735, USA. Email: aambi@enzo.com,

In particular, the levels of lipopolysaccharide (LPS) present in Gram-negative bacteria are notably elevated in the blood and brain in AD patients [6, 7]. LPS, or endotoxin, has the potential to produce the features seen in AD pathology, including amyloid β and tau aggregation and neuropathology, as described in Brown's 'endotoxin hypothesis of neurodegeneration' [6]. This was corroborated with the human study conducted by the Marizzoni group, as they showed the elevated levels of plasma LPS correlate with cerebral amyloid burden on PET ($\rho \geq 0.32$) [7]. Conversely, they also show the levels of butyrate (a protective bacterial metabolite) in blood have an inverse relationship relative to the presence of amyloid [7].

Although the downstream effects of both elevated LPS and reduced butyrate are increasingly understood, the upstream triggers to cause the changes in the gut permeability and the subsequent endotoxemia are largely unexplored. Brown and colleagues' seminal hypothesis explore the downstream drivers of neurodegeneration through the elevated concentration of LPS [6]. In addition, the recent comprehensive analysis linking LPS to AD pathology has provided further support for the endotoxin hypothesis [8]. Building on this foundational work characterizing the downstream effects of LPS in AD pathology, the next step is to determine the upstream trigger that initiates this gut dysbiosis in the aging population.

Interestingly, copper homeostasis is progressively altered with aging across multiple compartments. Brain copper levels increase in an age-dependent manner alongside upregulation of copper regulatory proteins in the subventricular zone and choroid plexus [9], and longitudinal PET/CT imaging confirms altered copper fluxes in the aging rodent brain [10]. At the systemic level, plasma copper accumulates with aging through mechanisms that remain incompletely understood [11], and recent activity-based sensing has demonstrated that elevated labile (not total) copper specifically drives liver aging through depletion of hepatic ALDH1A1 [12]. Importantly, it is the labile and non-ceruloplasmin-bound copper fractions, rather than total serum copper, that are biologically active and consistently elevated in AD [13]; the Cu:Zn ratio increases progressively across the adult lifespan and the redox-active copper content of ceruloplasmin increases twofold with age [14, 15], further reflecting dysregulation of copper handling. Copper is additionally a potent antimicrobial agent with well-characterized toxicity to anaerobic bacteria [16]. This positions copper as a candidate upstream trigger linking age-related metabolic changes to the gut dysbiosis described above. A meta-analysis notably confirmed that non-ceruloplasmin-bound free copper is consistently elevated in AD patients [13]. Subsequently, Squitti et al. have further shown that this elevation predicts the future exacerbation from mild cognitive impairment to full-blown dementia [17]. This shows the elevation of free copper levels precedes the clinical conversion in patients with AD. Regardless, a recent preliminary Mendelian Randomization (MR) study reported an association between genetically predicted copper levels and increased AD risk (OR = 1.291), though the mediation through gut microbiome pathways did not reach statistical significance and the findings should be interpreted cautiously given the limitations of the underlying GWAS [18]. In a separate study, Li et al. indicated that short-chain fatty acid (SCFA)-producing gut bacteria are protective against AD [19]. The question then arises whether copper might be affecting these beneficial microbes to subsequently influence AD risk.

We propose that age-related copper dyshomeostasis triggers gut microbiome dysbiosis by selectively eliminating anaerobic butyrate-producing bacteria. This consequently shifts the SCFA profile towards an increased acetate/butyrate ratio while simultaneously increasing gut permeability and systemic LPS translocation, creating a triple inflammatory insult that accelerates AD pathology. The acetate/butyrate ratio has been identified as a promising preliminary biomarker for AD in an initial cross-sectional study [20]. Our hypothesis provides an upstream mechanistic explanation for this observed effect (Fig. 1). Critically, no integrated human study has yet simultaneously demonstrated the full proposed sequence: age-related free copper elevation, selective loss of butyrate-producing taxa, acetate/butyrate ratio shift, endotoxemia, BBB disruption, and AD neuroinflammation. The evidence supporting each node of this pathway is assembled from independent studies across separate research domains, and the integrated framework remains to be validated in a single cohort.

This manuscript constitutes a narrative review in which literature was identified through targeted searches of PubMed and Google Scholar using terms including copper dyshomeostasis, gut-brain axis, Alzheimer's disease, butyrate, lipopolysaccharide, Wilson's disease, and short-chain fatty acids, supplemented by citation tracking from key foundational papers. Evidence was synthesized to construct a mechanistic hypothesis rather than to provide a systematic or exhaustive review of any single domain. The evidentiary strength of each component of the proposed pathway varies considerably, and throughout this manuscript we have endeavored to distinguish between direct human AD evidence, human analogies such as Wilson's disease, animal model data, in vitro mechanistic findings, and speculative or extrapolated links.

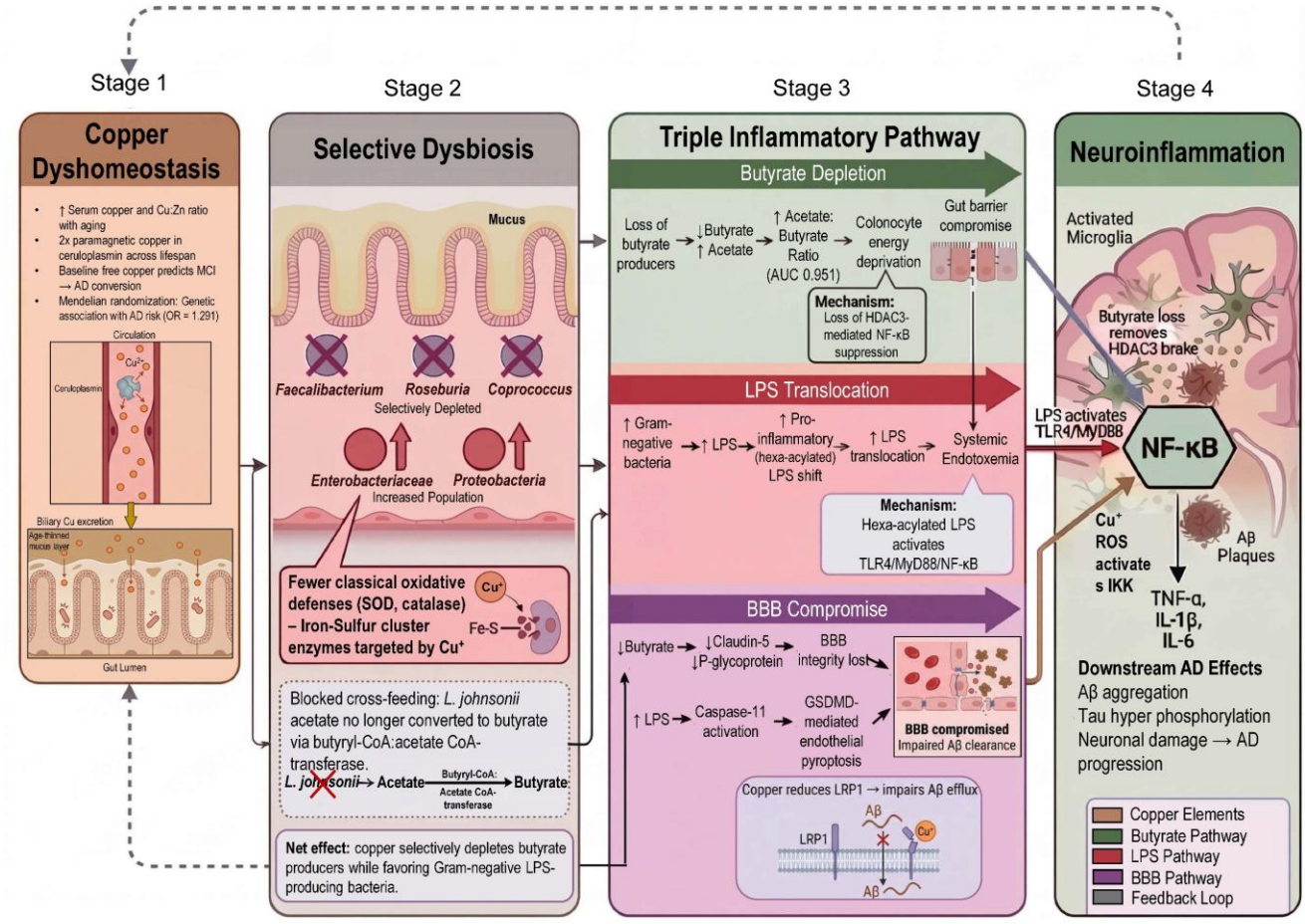

Figure 1. The copper-gut-brain axis: a proposed triple inflammatory pathway driving neuroinflammation in Alzheimer's disease. Stage 1: Age-related copper dyshomeostasis increases systemic free copper, which reaches the gut lumen via biliary excretion through an age-thinned mucus layer. **Stage 2:** Copper selectively depletes strictly anaerobic butyrate-producing bacteria (*Faecalibacterium*, *Roseburia*, *Coprococcus*) via Cu^{+}-mediated iron-sulfur cluster damage while favoring Gram-negative *Enterobacteriaceae* and *Proteobacteria*, blocking the *L. johnsonii*-dependent acetate-to-butyrate cross-feeding pathway. **Stage 3:** Triple inflammatory pathway comprising butyrate depletion (loss of HDAC3-mediated NF-κB suppression; acetate:butyrate ratio AUC 0.951), LPS translocation (hexa-acylated LPS activates TLR4/MyD88/NF-κB), and BBB compromise (↓claudin-5, ↓P-glycoprotein; caspase-11/GSDMD-mediated endothelial pyroptosis; copper-mediated LRP1 reduction impairing Aβ efflux). **Stage 4:** Triple convergence on NF-κB in which butyrate loss removes the HDAC3 brake, LPS activates TLR4/MyD88, and Cu^{+}-generated ROS activates IKK, driving TNF-α, IL-1β, and IL-6 production and downstream AD pathology, including Aβ aggregation and tau hyperphosphorylation. Dashed arrow indicates bidirectional feedback between neuroinflammation and copper metabolism.

Evidence for Copper Dysregulation in AD

As we established copper as a potential upstream candidate, we now examine the specifics of this association in detail. The elevation of non-ceruloplasmin-bound ("free") copper in AD has been replicated across multiple independent studies and methodologies [13, 21–23]. Bucossi et al.'s meta-analysis of serum and plasma studies confirmed significantly higher copper levels in AD patients compared to controls [21] building on previous work correlating copper with cognitive decline [22] and earlier clinical characterization of non-ceruloplasmin copper excess in AD [23]. Critically, free copper elevation is not merely a single-timepoint observation, as Squitti et al. showed that baseline free copper levels predict future cognitive decline, suggesting copper elevation precedes rather than follows cognitive decline [17]. The age-related increase in the Cu:Zn ratio [14] positions copper dysregulation as a feature of normal aging that becomes problematic in AD-vulnerable individuals.

The root cause of the pathogenic effects of elevated free copper levels can be explored through investigating the molecular mechanisms of its toxicity. Studies have

characterized a copper-dependent regulated cell death as "cuproptosis," which is distinct from apoptosis and ferroptosis [24]. Tsvetkov et al. recently revealed that excess copper directly binds to lipoylated components of the tricarboxylic acid cycle, triggering protein aggregation and mitochondrial dysfunction through Ferredoxin1 (FDX1)-dependent pathways [24]. This cuproptosis mechanism has been extended to neurodegeneration, where copper-induced disruption of mitochondrial metabolism through lipoylated protein aggregation has been implicated in AD pathology [25]. Okafor et al. noted that copper toxicity is not uniform across cell types and specifically showed patterns of copper dyshomeostasis in AD brain tissue [26]. This selective copper pathology has been mapped in detail in the CNS; however, similar differential effects of copper on gut microbial communities are largely unexplored, despite the metabolic and redox environment of the gut potentially creating distinct microbial vulnerability patterns. This distinction between copper fractions is mechanistically significant: labile and non-ceruloplasmin-bound copper, rather than total or ceruloplasmin-bound copper, represents the biologically active species capable of generating reactive oxygen species and exerting selective antimicrobial pressure in the anaerobic gut environment.

Complementing the empirical evidence, MR studies, which leverage genetic variants associated with an exposure as instruments to infer causality, have provided preliminary genetic associations between copper and AD risk, though causal interpretation remains limited. Li and colleagues reported that genetically predicted copper levels are associated with increased AD risk (OR = 1.291, IVW method) with some evidence suggesting gut-mediated pathways may contribute to this association [18]. In a separate MR study, Li et al. found that abundance of SCFA-producing gut bacteria is associated with reduced AD risk [19]. Taken together, these MR studies raise the possibility that copper elevation may affect SCFA-producing gut bacteria and thereby influence AD vulnerability; however, this interpretation requires careful qualification. Interestingly, while the overall copper-AD association reached significance (OR = 1.291), this finding is more accurately interpreted as reflecting genetically determined variation in copper metabolism broadly, rather than a direct effect of any specific copper fraction or pool. The underlying component paths are worth noting: statistically significant associations were identified between copper and the lipid IVA biosynthesis pathway and between this pathway and AD risk individually; however, the overall mediation through gut microbiota pathways did not achieve statistical significance. This pattern, in which individual component paths are significant, yet the indirect effect is not, precludes strong mechanistic conclusions about gut involvement and should be considered hypothesis-generating rather than directional evidence of a gut-mediated mechanism. Furthermore, the exposure GWAS was substantially underpowered (n = 2,603) with only two genome-wide significant instruments, and critically, the GWAS measured erythrocyte copper rather than non-ceruloplasmin-bound free copper, which is the specific fraction consistently implicated in AD by observational meta-analyses [13, 21–23]. These results should therefore be considered hypothesis-generating rather than directional evidence of causality and require replication in larger, well-powered cohorts using instruments derived from adequately powered GWAS measuring the appropriate copper fraction [18].

Proposed Mechanism

Copper promotes toxicity through multiple mechanisms, including the generation of reactive oxygen species (ROS) through Fenton-like chemistry [16]. Interestingly, Outten et al. showed that copper toxicity is maximized under anaerobic conditions since the more toxic Cu(I) state is more stable and key detoxification enzymes such as multicopper oxidases remain inactive without dioxygen [16, 27]. This is particularly relevant to obligate anaerobes, which rely on iron-sulfur cluster enzymes such as pyruvate:ferredoxin oxidoreductase (PFOR) for central carbon metabolism. These enzymes are directly inactivated by molecular oxygen [28], and their exposed iron-sulfur clusters represent primary intracellular targets of copper toxicity [29]. Obligate anaerobes are thus doubly constrained: they cannot tolerate the aerobic environments where copper toxicity is diminished, nor can they protect the iron-sulfur enzymes essential to their metabolism from copper-mediated damage. It has been reported that butyrate-producing species such as Faecalibacterium prausnitzii are extremely oxygen-sensitive, though they can survive at oxic-anoxic interfaces through extracellular electron shuttling [30, 31]. These organisms are thus vulnerable to copper toxicity on multiple fronts: they cannot exert effective oxygen-dependent copper defenses, cannot escape to aerobic niches where copper toxicity would be diminished and appear to possess fewer characterized copper resistance mechanisms compared to aerotolerant organisms [16].

The structure of the biosynthetic pathways amplifies the vulnerability of butyrate production due to copper. There are four main bacterial butyrate synthesis pathways, including the acetyl-CoA, glutarate, 4-aminobutyrate, and lysine pathways, among which the acetyl-CoA pathway is overwhelmingly dominant in the human gut, and it accounts for the majority of butyrate production (~80%

prevalence) across diverse microbial communities [32, 33]. This specific pathway is heavily dependent on the microbial cross-feeding initiated by acetate-producing bacteria (Lactobacillus and Bifidobacterium species) to supply acetate substrate that is subsequently converted to butyrate by Faecalibacterium, Roseburia, and Coprococcus via the butyryl-CoA:acetate CoA-transferase enzyme [32]. This reliance on microbial cross-feeding could be a source of vulnerability in the downstream pathway and potentially block the butyrate production. Wen and colleagues demonstrated this vulnerability when copper exposure eliminated Lactobacillus johnsonii, the primary acetate supplier, resulting in acetate accumulation and butyrate deficiency [34]. Importantly, since the acetyl-CoA pathway uses acetate as both substrate and co-product, disruption of the microbial cross-feeding explains the observation of an increase in acetate levels while a decrease in butyrate levels in AD patients [20]. The structural dependence of butyrate production on intact cross-feeding networks thus provides the mechanistic explanation for why copper-driven dysbiosis produces this specific metabolic signature rather than a generalized decline in all SCFAs.

Copper sensitivity varies between the bacterial species considerably, hence creating a selective pressure that affects the microbiome composition. While strict anaerobes are highly vulnerable, certain Lactobacillus and Lactococcus species have copper resistance mechanisms that can survive even under elevated copper conditions [35]. Overall, these studies collectively suggest that copper elevation would selectively eliminate vulnerable butyrate producers while sparing resistant organisms and ultimately reshaping the gut microbiome. Particularly, this pattern favors Gram-negative bacteria at the expense of strictly anaerobic Firmicutes [34, 36].

Independent studies corroborate this copper-dysbiosis mechanism. A separate study has validated this finding independently, where Fernandez and colleagues found that copper exposure decreased lactic acid bacteria while increasing Enterobacteriaceae, and again, these effects reversed upon copper removal [36]. Recently, Wen and colleagues provided further confirmation that dietary copper-driven colonic dysbiosis drives oxidative stress and butyrate deficiency in pigs [37]; this strengthens the overall copper-dysbiosis-butyrate pathway. Similarly, a recent review of the literature on metal-microbiota interactions confirms that dietary copper reduces the abundance of butyrate-producing genera, including Faecalibacterium and Roseburia [38]. Long-term metal exposure studies further confirm that chronic exposure leads to lasting changes in the gut microbiota composition [39]. Although these studies used supraphysiological copper concentrations, they establish the mechanistic principle that copper can selectively eliminate butyrate-producing bacteria.

Wilson's disease (WD) is a genetic disorder of copper accumulation and, in this context, provides a human proof-of-concept that endogenous copper overload disrupts gut homeostasis. Multiple independent studies have characterized the changes in the gut microbiome in Wilson's disease patients and consistently show significant dysbiosis [40–43]. The most comprehensive and recent analysis to date used the shotgun metagenomic sequencing method in 37 WD patients and 33 controls and confirmed significant differences in gut microbial composition [43]. Across studies, a consistent finding is the depletion of butyrate-producing taxa, which includes the specific reduction of Roseburia inulinivorans [42] and decreased abundance of Lachnospiraceae, Ruminococcaceae, and Coprococcus [41]. Cai and colleagues found altered Firmicutes/Bacteroidetes ratios in WD patients [41], and their subsequent multi-omics analysis revealed that WD-associated dysbiosis includes altered bile acid metabolites [42]. The most recent shotgun metagenomic analysis by Wei et al. additionally identified increased abundance of virulence factors and antimicrobial resistance genes in WD gut microbiota, indicating that copper overload selects for more pathogenic and resistant microbial communities beyond the simple loss of butyrate producers [43]. Although phylum-level changes are not consistent across the cohorts, the selective loss of butyrate-producing genera is a common finding and is consistent with the pattern expected from copper-mediated toxicity to strict anaerobes. Notably, most WD patients in these studies were receiving copper-lowering therapy (D-penicillamine or zinc), which may independently influence microbiome composition; however, the consistent depletion of butyrate-producing genera across studies with different treatment regimens suggests copper overload itself rather than treatment drives this pattern. Interestingly, fecal microbiota transplantation (FMT) of healthy microbiota in WD mouse models reduced hepatic copper concentration and alleviated liver injury, suggesting bidirectional gut-copper relationships [44]. Gut microbiota diversity was similarly reduced [45], suggesting that systemic copper overload rather than local intestinal copper transport drives the dysbiosis. WD represents a substantially elevated case of copper overload of up to approximately 25-fold above normal hepatic copper levels, and it is important to note that WD is a rare monogenic disorder with hepatic, biliary, neurological, and treatment-related effects that differ fundamentally from the gradual, systemic copper accumulation of normal aging and sporadic AD. Whether the considerably milder elevations observed in normal aging produce

analogous effects on the gut microbiome requires direct investigation and cannot be assumed from WD data alone.

The proposed copper-gut-brain mechanism is supported by parallel findings with other transition metals. For instance, Liu et al. demonstrated that FMT successfully reversed manganese-induced neurotoxicity through modulation of cGAS-STING and NLRP3 inflammatory pathways [46]. Iron similarly has been implicated in causing dysregulation, which impacts neurodegeneration through gut-mediated mechanisms involving hepcidin signaling [47]. More broadly, cadmium and lead exposure have been shown to disrupt gut microbiota composition, reduce SCFA levels, and increase LPS production in animal models [38]. The consistent pattern across multiple metals with dysbiosis, reduced SCFA production, increased gut permeability, and downstream neuroinflammation overall suggests that metal-induced gut dysbiosis may represent a general mechanism linking environmental and age-related metal exposures to neurodegeneration. Among these metals, copper stands out for its well-characterized elevation in both normal aging [14, 15] and AD [13]. While iron dysregulation has been proposed as a similar metal-mediated trigger of gut dysbiosis in neurodegeneration, copper has the distinct advantage of established meta-analytic evidence for elevated free copper specifically in AD patients [13], longitudinal prediction of MCI-to-AD conversion [17], and MR support for a causal direction [18]. Similar levels of evidence are still scarce for iron.

Table 1. Evidentiary hierarchy for each node of the proposed copper-gut-brain axis hypothesis. Evidence types are categorized as direct human AD evidence, indirect human analogy (Wilson's disease), animal model evidence, in vitro or microbiology data, and speculative or extrapolated links. Strength designations reflect the current state of the literature rather than the plausibility of the proposed mechanism.

Pathway Node	Proposed Link	Evidence Type	Key Reference(s)	Strength
Copper increases with aging	Serum and tissue copper accumulate progressively; labile fraction increases in liver and brain	Human and animal aging data	Fu et al. [9]; Peng et al. [10]; Wang et al. [11]; Zhao et al. [12]; Piacenza et al. [14]; Musci et al. [15]	**Established**
Free copper elevated in AD	Non-ceruloplasmin-bound copper elevated in AD; predicts MCI-to-AD conversion	Direct human AD evidence	Squitti et al. [13]; Bucossi et al. [21]; Squitti et al. [17]	**Established**
Copper causally linked to AD risk	Genetically predicted copper associates with AD risk; gut-mediated pathway not statistically confirmed	Mendelian randomization	Li Y et al. [18]; Meng et al. [78]; Evans et al. [79]	**Hypothesis -generating**
Copper selectively toxic to anaerobes	Cu(I) inactivates iron-sulfur cluster enzymes; toxicity maximal under anaerobic conditions	In vitro and microbiology data	Outten et al. [27]; Macomber & Imlay [29]; Lu & Imlay [28]; Dupont et al. [16]	**Established (mechanistic)**
Copper depletes butyrate producers	Dietary copper eliminates butyrate-producing genera; reversed on copper removal	Animal model (supraphysiological doses)	Wen et al. [34]; Wen et al. [37]; Fernandez et al. [36]; Zhu et al. [38]	**Preliminary**
Endogenous copper overload disrupts microbiome	Consistent depletion of butyrate-producing genera in Wilson's disease across independent cohorts	Human analogy (Wilson's disease)	Cai et al. [41]; Cai et al. [42]; Wei et al. [43]; Geng et al. [40]	**Moderate**
Dysbiosis shifts acetate/butyrate ratio	Copper disrupts cross-feeding networks, producing the SCFA signature observed in AD	Animal model + preliminary human AD data	Wen et al. [34]; Marizzoni et al. [20]	**Preliminary**
Butyrate loss activates NF-kB	Butyrate inhibits HDAC3, suppressing NF-kB; depletion removes this brake	In vitro and animal model evidence	Parada-Venegas et al. [60]; Sun et al. [63]; Erny et al. [61]; Mishra et al. [62]	**Preliminary**
LPS activates TLR4/NF-kB	Plasma LPS correlates with amyloid burden in humans; hexa-acylated LPS activates TLR4	Direct human AD + mechanistic data	Marizzoni et al. [7]; Brown [6]; Brown & Heneka [8]; d'Hennezel et al. [55]	**Established**

BBB compromise reduces Ab clearance	Butyrate loss reduces claudin-5; LPS activates GSDMD pyroptosis; copper reduces LRP1	In vitro and animal model; unreviewed preprint*	Wei et al. [58]; Li YQ et al. [59]; Veerareddy et al. [57]* (unreviewed preprint)	**Preliminary**
Integrated pathway drives AD	Age-related copper elevation initiates selective dysbiosis and triple NF-kB convergence	Speculative — no integrated human study	Hypothesis only	**Hypothesis -generating**

The relationship between copper and the gut is bidirectional. Antibiotic-induced decrease in gut microbiota diminished the expression of copper transporters in the colon [48]. Separately, FMT intervention reduced the hepatic copper concentration and alleviated liver injury in Wilson's disease animal models [44]. Therapeutic interventions using Akkermansia supplementation alongside D-penicillamine led to decreased copper levels, demonstrating the therapeutic value in such therapies [49]. Feng et al. demonstrated in a randomized controlled trial that probiotic supplementation in occupationally exposed workers reduced blood copper levels by over 34% and simultaneously increased fecal SCFA production; furthermore, complementary mouse experiments showed that this protective effect was negated when the gut microbiota was pre-treated with antibiotics [50]. While this study examined toxic occupational copper exposure rather than age-related elevation, the mechanistic principle of microbiota-mediated copper reduction is relevant to our framework.

Downstream Consequences — Triple Pathway

Butyrate depletion initiates the first arm of the triple inflammatory pathway. Butyrate is the primary energy source for colonocytes, and it is essential for maintaining gut barrier integrity through upregulation of tight junction proteins, including claudins, occludin, and zonula occludens-1 [51]. In addition, butyrate also acts as a strong histone deacetylase inhibitor and consequently, promotes anti-inflammatory gene expression while suppressing pro-inflammatory cytokine production in intestinal epithelial cells [51]. Colonocytes prefer butyrate as a metabolic substrate; however, during butyrate scarcity, they switch to glucose oxidation, a metabolic change that itself promotes a pro-inflammatory state [51]. Copper-induced depletion of butyrate-producing bacteria may therefore undermine each of these protective functions. Consistent with this, animal studies report gut barrier damage following copper exposure [34], suggesting that the resulting intestinal permeability could contribute to downstream systemic inflammatory signaling.

The second arm of the triple inflammatory pathway is through LPS translocation driven by the increased Gram-negative bacterial abundance described above. This event of mobilization of LPS is the initial trigger in Brown's endotoxin hypothesis of neurodegeneration [6], which has been comprehensively updated by Brown and Heneka, linking LPS specifically to Alzheimer's disease pathology [8]. Human studies confirm this relationship: plasma LPS levels correlate significantly with cerebral amyloid burden ($\rho \geq 0.32$), while butyrate shows an inverse and protective relationship [7]. Lipopolysaccharide binding protein (LBP), a more stable marker of endotoxin exposure compared to the rapidly cleared LPS, is elevated in AD patients carrying the APOE3/E3 genotype, providing additional human evidence for gut-derived LPS translocation [52]. Our hypothesis provides a potential upstream mechanistic explanation for these observations. Copper-driven elimination of butyrate-producing bacteria may shift the microbial composition toward LPS-producing Gram-negative species while also compromising gut barrier integrity, together creating conditions that could favor chronic endotoxemia.

The LPS group is structurally diverse, and not all LPS species are equally inflammatory. The immunogenicity of LPS is determined by the acylation pattern of its lipid A domain, and variation in this structure across bacterial taxa has been shown to have direct clinical consequences [53]. For instance, d'Hennezel et al. demonstrated that total fecal LPS from healthy humans is immunoinhibitory; it is driven by underacylated LPS produced by members of the order Bacteroidales, which antagonizes TLR4-MD2 signaling, effectively silencing inflammatory responses [54]. In contrast, LPS from Proteobacteria such as Escherichia coli is hexa-acylated and activates TLR4 and downstream NF-κB inflammatory cascades [53, 54]. The selective dysbiosis pattern predicted by our copper hypothesis (↓ Firmicutes and ↑ Proteobacteria) not only increases total LPS availability but also shifts the LPS pool toward more inflammatory species. This critical nuance explains why microbiome composition changes may have greater inflammatory impact than absolute LPS levels alone.

Copper-induced dysbiosis also compromises the blood-brain barrier (BBB), creating a third arm of the inflammatory pathway. Recent evidence suggests that BBB dysfunction may precede classical AD pathology [55], positioning it as an early target for the upstream copper-induced dysbiosis proposed here. Butyrate upregulates the tight junction protein claudin-5 and the efflux transporter P-glycoprotein to maintain the integrity

of the BBB. Veerareddy and colleagues reported in an unreviewed preprint that butyrate may restore BBB-mediated amyloid-β clearance in endothelial cells through activation of the insulin-signaling pathway [56]; however, as this finding has not yet undergone peer review, it should be treated as preliminary, and the mechanistic conclusions drawn from it remain tentative pending independent replication. In contrast, LPS directly damages the BBB through activation of caspase-11 and gasdermin D (GSDMD) in brain endothelial cells, which triggers pyroptotic cell death and compromises the BBB [57]. In addition, copper further compromises BBB integrity, as free copper enters the brain more readily than protein-bound copper and has been shown to reduce LRP1 expression, thereby impairing the receptor-mediated clearance of amyloid-β across the BBB [58]. Together, butyrate loss, LPS-mediated damage, and copper-driven LRP1 reduction may each independently compromise BBB function. If these effects co-occur, they could facilitate entry of peripheral inflammatory mediators into the CNS and impair amyloid-β clearance, potentially reinforcing a feed-forward loop between peripheral dysbiosis and central neurodegeneration (Fig. 1).

The copper-induced dysbiosis thus creates a triple inflammatory insult that converges on a single molecular target: NF-κB. The first convergence arm involves the loss of butyrate-mediated NF-κB suppression. Butyrate is a potent histone deacetylase inhibitor, and recent evidence has identified HDAC3 as the specific isoform through which butyrate exerts its anti-inflammatory effects in monocytes and macrophages, increasing H3K9 acetylation and suppressing TNF-α and IL-6 production [59]. Within the CNS, microglia similarly depend on SCFA for proper maturation and homeostatic function [60], and importantly, Mishra et al. demonstrated that the age-related decline in butyrate-producing bacteria led to brain inflammation and cognitive decline in aging mice through suppressed FFAR2/3 signaling; butyrate supplementation reversed these effects [61]. The second convergence arm is through LPS-mediated NF-κB activation. Translocated LPS binds TLR4 on microglia, activating the MyD88-dependent signaling cascade that phosphorylates NF-κB p65 and drives pro-inflammatory cytokine transcription [6]. Critically, Sun and colleagues demonstrated that sodium butyrate attenuates this exact pathway, significantly reducing phosphorylated NF-κB p65, MyD88, and TLR4 proteins in both brain tissue and BV2 microglia [62]. This is particularly significant because it shows that butyrate directly suppresses the same NF-κB signaling cascade that LPS activates; copper-induced butyrate loss therefore removes this brake at precisely the moment that increased LPS translocation presses the accelerator. The third convergence arm involves copper itself. At sub-neurotoxic concentrations, copper(II) has been shown to generate mitochondrial ROS and enhance IκB kinase (IKK) activity, with downstream effects on NF-κB-dependent microglial activation and pro-inflammatory cytokine transcription, including TNF-α, IL-1β, and IL-6 [63].

Acetate/Butyrate Ratio as Mechanistic Linchpin

The acetate/butyrate ratio has recently been identified as a biomarker distinguishing cognitive impairment due to AD from cognitive impairment of other etiologies. Marizzoni and colleagues demonstrated that this ratio achieves an area under the receiver operating characteristic curve of 0.951 (95% CI: 0.885–1.000) for distinguishing cognitive impairment due to AD (CI-AD) from cognitive impairment not due to AD (CI-NAD) [20]. While this preliminary finding is promising, the study included only 67 participants with a heterogeneous comparison group, and the authors acknowledge the need for validation in larger cohorts. Direct comparison with extensively validated biomarkers such as GFAP is therefore premature at this stage. Specifically, CI-AD patients showed significantly higher acetate and valerate and lower butyrate levels compared to both CI-NAD and cognitively unimpaired individuals, indicating that this SCFA profile shift is specific to AD-related cognitive impairment rather than cognitive decline in general [20]. This finding positions the acetate/butyrate ratio as a candidate molecular signature of AD-related gut dysfunction pending replication. This is precisely the SCFA profile shift predicted by copper-mediated selective targeting of butyrate-producing anaerobes, which subsequently decreases butyrate production with preserved or increased acetate from resistant bacteria.

Wen and colleagues provided the direct mechanistic basis for this altered ratio. Their study demonstrated that copper exposure eliminated key members of the microbial cross-feeding network through which Lactobacillus johnsonii-derived acetate is converted to butyrate via the butyryl-CoA:acetate CoA-transferase pathway [32, 34]; consequently, acetate accumulated while butyrate levels declined. This disrupted conversion mechanism accounts for the specific SCFA signature observed in AD patients, where acetate levels are elevated and butyrate levels are diminished simultaneously rather than a general reduction in all SCFAs [20]. Our hypothesis thus provides a specific, testable upstream mechanism for an already-identified diagnostic signature and represents the central contribution of this work by identifying copper as the trigger that initiates the metabolic disruption underlying the acetate/butyrate shift in AD.

It is important to note that the relationship between SCFAs and AD pathology is context-dependent. Interestingly, Colombo et al. demonstrated that SCFA

supplementation in germ-free APP/PS1 mice increased amyloid plaque deposition and upregulated the ApoE-TREM2 microglial axis rather than providing neuroprotection [64]. Similarly, Seo et al. showed that SCFA supplementation in a tauopathy mouse model induced hippocampal gliosis and tau pathology in a microbiota- and ApoE isoform-dependent manner [65]. These findings underscore a critical distinction that exogenous SCFA supplementation does not address the potential root cause of the absence of normal microbiome diversity, which could endogenously produce the necessary SCFAs. Our hypothesis specifically addresses the endogenous shift in SCFA profiles driven by copper-induced dysbiosis, not exogenous supplementation, and this distinction explains why the acetate/butyrate ratio captures disease-relevant changes that absolute SCFA levels may miss.

Testable Predictions

First, a cross-sectional study in aging adults without dementia should simultaneously measure serum free copper, fecal microbiome composition, plasma and fecal SCFA profiles, and markers of endotoxemia (LPS and LBP) in the same cohort. Our hypothesis predicts that serum free copper levels should correlate positively with the fecal acetate/butyrate ratio and plasma LPS/LBP levels while inversely correlating with the abundance of strictly anaerobic butyrate-producing genera, including Faecalibacterium, Roseburia, and Coprococcus. Importantly, this study should stratify participants by age decade to determine whether these correlations strengthen with advancing age, as predicted by the progressive increase in Cu:Zn ratio and redox-active copper across the lifespan [14, 15]. This would provide the first integrated test of the proposed copper-gut-brain pathway in a single human cohort, directly addressing the current gap where copper dysregulation and gut dysbiosis have been studied independently but never together. As larger GWAS for serum copper become available, MR analyses testing the causal relationship between copper levels and butyrate-producing bacteria using microbiome data from consortia such as MiBioGen will become feasible and represent a priority for genetic validation of this hypothesis.

Second, copper-lowering therapeutic interventions should shift gut microbiome composition toward increased population of butyrate-producing microbiota, including Faecalibacterium, Roseburia, and Coprococcus, while reducing the fecal acetate/butyrate ratio and decreasing plasma LPS and LBP levels. An interventional study measuring fecal microbiome composition and SCFA profiles before and after copper-lowering therapy would directly test the proposed causal relationship between copper and gut dysbiosis. Moreover, the magnitude of butyrate-producing bacteria recovery should correlate with the degree of copper reduction. Importantly, if copper is the upstream trigger as proposed by our hypothesis, then reducing bioavailable copper should simultaneously reverse the selective dysbiosis pattern and its downstream inflammatory consequences. This specificity would distinguish our hypothesis from models in which gut dysbiosis in AD arises independently of copper status.

Third, a combined therapeutic approach targeting both copper reduction and microbiome restoration should provide greater efficacy than either approach alone. Our hypothesis predicts this superiority because copper chelation or dietary modification alone would remove the upstream trigger of copper dyshomeostasis but cannot immediately repopulate the depleted butyrate-producing communities. Similarly, FMT or targeted probiotics alone would restore these communities without addressing the ongoing copper-mediated selective pressure that caused their elimination. Evidence that probiotic supplementation improves cognition in AD patients [66] and that genetically predicted SCFA-producing bacteria are protective against AD [19] supports the therapeutic potential of microbiome restoration. Our framework predicts that a durable therapeutic benefit requires the simultaneous reduction of the copper burden that drives dysbiosis.

Fourth, in vitro studies should directly address the dose-extrapolation limitation of existing animal models by demonstrating that physiologically relevant copper concentrations can produce selective toxicity to strictly anaerobic butyrate-producing bacteria. Specifically, anaerobic cultures of Faecalibacterium, Roseburia and Coprococcus exposed to copper concentrations reflecting elevations observed in aging should show dose-dependent reductions in both viability and butyrate output, with a corresponding increase in the acetate/butyrate ratio of the culture media, while the copper-resistant bacteria remain unaffected. This experiment is essential because current evidence for copper-mediated selective dysbiosis derives from animal studies using supraphysiological copper doses [34], and the relevance to human aging remains unclear. If strict anaerobes can tolerate copper concentrations representative of aging-related elevations without significant growth inhibition or metabolic disruption, the central crux of our hypothesis would require revision.

Fifth, our hypothesis predicts that sex disparities in AD prevalence may be partly due to copper status. Due to estrogen-mediated upregulation of ceruloplasmin synthesis, women's serum copper levels are about 20–30% higher than men's, and epidemiological data indicates that copper significantly increases the risk of AD, particularly in older women [14, 67]. Sex-stratified

analysis in the proposed study should reveal stronger correlations between copper, dysbiosis, and endotoxemia markers in women. This prediction is readily testable within the study design already outlined above and could help explain the well-documented female predominance in AD.

Therapeutic Implications

The AD drug development pipeline has endured decades of failed amyloid-targeting trials [68], and even approved therapies show limited cognitive improvement. If validated, the identification of copper dyshomeostasis as an upstream trigger would carry significant therapeutic implications. However, given the indirect nature of the current evidence base, the mixed and at times conflicting history of copper chelation approaches in AD and CAA models, and the absence of any clinical trial targeting systemic free copper in the context of gut dysbiosis, copper modulation should be considered a research direction requiring prospective validation rather than a clinical recommendation. The therapeutic discussion presented here is intended to identify testable interventional hypotheses, not to advocate for clinical implementation. Copper represents a measurable, modifiable target; serum free copper can be readily quantified, and copper-lowering strategies, including chelation therapy and dietary changes, are already clinically available. We previously evaluated copper chelation therapy using tetrathiomolybdate (TTM) in transgenic animal models of AD and cerebral amyloid angiopathy (CAA), which revealed conflicting results [69, 70]. The TTM treatment reduced copper accumulation in cerebral amyloid plaques in an AD mouse model [69], yet paradoxically increased amyloid-β load and copper colocalization with vascular amyloid deposits in a rat model of CAA [70]. These conflicting results highlight the compartmental complexity of copper in amyloid pathology across disease models, species, and routes of administration. More recently, novel copper chelators have been shown to reduce neuroinflammation and oxidative stress, restore copper homeostasis in the hippocampus of AD rat models, and improve spatial memory [71], suggesting that refined chelation approaches may overcome the limitations observed with earlier agents. This focus on brain copper chelation, despite its conflicting results and compartmental complexity, reinforces the rationale for our hypothesis: rather than chelating brain copper, targeting the upstream elevation of systemic free copper that drives gut dysbiosis may be a more precise therapeutic strategy. Importantly, intervening before irreversible neuronal damage has occurred may prove more effective than addressing downstream pathology. If systemic copper reduction is indeed the appropriate therapeutic target, the acetate/butyrate ratio reported by Marizzoni and colleagues [20] represents a candidate biomarker that, if validated in larger cohorts, could potentially be used to monitor whether copper-lowering interventions successfully reverse the downstream dysbiosis.

Microbiome-targeted therapeutic interventions provide a complementary route that directly addresses the downstream consequences of copper-induced dysbiosis. Randomized controlled trials have shown significant cognitive improvements with probiotic treatment in AD patients, including Mini-Mental State Examination (MMSE) gains with single-strain probiotic formulations [66]. Earlier trials have also examined multi-strain probiotic formulations in AD, though results in more severe disease stages were less consistent [72]. Within our framework, these benefits are consistent with the partial restoration of butyrate-producing communities and reduced gut-derived neuroinflammation. Importantly, Akkermansia supplementation, for instance, has been shown to enhance copper removal not only from the liver but also from the brain during chelation therapy in a Wilson's disease model [49], representing preliminary evidence, in a Wilson's disease model, that microbiome modulation may influence copper handling. Whether analogous effects occur in the context of age-related copper elevation in AD remains to be established. Whether such microbiome-targeted approaches could complement copper-lowering strategies in AD remains to be directly tested, but the convergence of these independent lines of evidence suggests this combination warrants investigation.

Limitations

The most important and unresolved biological question for this hypothesis is whether the modest copper elevations observed in normal aging are sufficient to exert the selective antimicrobial pressure on butyrate-producing anaerobes proposed here. Current mechanistic evidence derives predominantly from supraphysiological copper doses in animal models, occupational copper exposure, or Wilson's disease, all of which differ materially from typical aging-associated copper changes. While the arguments presented in this manuscript, including the labile copper fraction, age-related mucus layer thinning, and cumulative low-dose exposure over decades, provide plausible reasons why modest elevations may produce disproportionate effects, this remains the central unresolved question, and the findings should be interpreted accordingly. Mechanistic studies presented by Wen and colleagues used copper supplementation in pigs

[34], and an independent study corroborated these findings using copper doses that exceed normal human dietary exposure [36]. Wilson's disease studies confirm that endogenous copper overload, which ranges up to ~25-fold above normal hepatic levels, is common [73]. It is currently unclear whether copper elevations in normal aging can produce similar levels of selective dysbiosis, and this remains the central unresolved question for our hypothesis. Controlled pig feeding trials (n=120–200) found that overall microbiome composition was relatively resistant to dietary copper supplementation, though specific reductions in Lactobacillus were observed [74, 75]. Our hypothesis suggests that exposure to minimally elevated copper in human aging for decades can produce cumulative selective effects that cannot be replicated in short-term animal studies [14]. Several features of the aging gut may increase the antimicrobial impact of copper beyond what acute-exposure studies predict. Intestinal mucins present within the mucus layer regulate copper absorption and modulate the extent of transport of copper ions to the underlying epithelium and microbes [76]. Age-induced thinning of the mucus layer would therefore increase the level of copper exposure to the microbes regardless of the changes in systemic copper levels. Furthermore, age-related loss of intestinal barrier integrity has been shown to be due to microbiota rather than a consequence of aging itself [77]. This suggests that initial copper-mediated dysbiosis could trigger a self-reinforcing cycle: copper eliminates protective bacteria, barrier integrity declines, copper bioavailability increases, and further dysbiosis continues. These levels of damage caused by this loop over decades could explain why the modest copper elevations observed in normal aging produce disproportionate effects on the gut microbiome later in life. Moreover, the underlying antimicrobial mechanism of copper toxicity involves generating ROS through Fenton-like chemistry [16], operating in a concentration-dependent manner. Strictly anaerobic bacteria possess fewer oxidative defense mechanisms, such as superoxide dismutase and catalase [28], and would therefore have lower copper tolerance compared to aerotolerant species. This suggests that even modest copper elevations could selectively affect oxygen-sensitive butyrate producers. The in vitro experiments in our testable predictions are designed to address this critical gap.

The Mendelian randomization evidence relating copper to AD risk has important methodological limitations that preclude strong causal conclusions. The major concern is the exposure GWAS, which included only 2,603 participants, which substantially limits the statistical power and instrument strength [18]. The mediation analysis suggesting gut pathway involvement produced confidence intervals that crossed zero, indicating that the lack of statistical significance should be considered hypothesis-generating rather than causal evidence. Replication in larger, well-powered cohorts with stronger genetic instruments is essential before causal claims can be confirmed. MR studies of copper and AD rely on the same small exposure GWAS (n = 2,603) measuring erythrocyte copper rather than the non-ceruloplasmin-bound free copper specifically implicated in AD by observational meta-analyses. This has produced contradictory findings, with different analytical choices yielding both risk-increasing and protective associations from identical genetic instruments. Until adequately powered GWAS datasets measuring serum free copper become available, MR approaches cannot meaningfully evaluate the specific copper fraction central to our hypothesis [18,78,79]. Regardless, the directional consistency of the MR findings with the extensive observational evidence from Squitti and colleagues, who have demonstrated elevated free copper in AD across multiple independent cohorts [13, 17, 21–23], provides comprehensive support for the copper-AD relationship even in the absence of definitive genetic proof. As large-scale biobank studies increasingly incorporate trace metal measurements, adequately powered copper GWAS datasets should become available to resolve this limitation.

The relationship between SCFAs and AD pathology is context-dependent and warrants careful interpretation. Colombo and colleagues demonstrated that exogenous SCFA supplementation in germ-free APP/PS1 mice paradoxically increased amyloid plaque deposition and upregulated the ApoE-TREM2 microglial axis [64]. This finding appears to contradict the protective role of butyrate that is central to our hypothesis. However, this contradiction reinforces an important distinction in our framework. Germ-free mice completely lack the microbial ecosystem that normally regulates SCFA production and cross-feeding networks. Delivering a concentrated dose of SCFAs into such a system bypasses this regulatory machinery entirely, whereas endogenous production within an intact microbiome provides continuous and physiologically regulated levels through established metabolic networks. Our hypothesis addresses the disruption of endogenous SCFA profiles driven by copper-induced dysbiosis rather than the supplementation of SCFAs into a system that lacks normal gut microbiome diversity. Hence, the acetate/butyrate ratio serves as a more meaningful indicator of disease-relevant changes than absolute SCFA levels alone since it directly reflects the integrity of the microbial cross-feeding networks that are disrupted by copper.

A critical limitation of evidence presented is the lack of any integrated study that simultaneously measures copper status, microbiome composition, SCFA profiles

and ratios, endotoxemia markers, and AD pathology in the same cohort. The evidence supporting our hypothesis is assembled from independent studies examining different components of the proposed pathway, each of which has been validated within their respective fields. Our hypothesis provides the conceptual framework that unifies these separate lines of evidence and identifies the specific measurements that need to be combined in a single cohort study. The cross-sectional study design proposed in our testable predictions is intended to address this gap and represents a feasible next step that can be implemented with existing methodologies and commercially available assays. Additionally, gut dysbiosis in AD is multifactorial since the increase in using medications (particularly cholinesterase inhibitors), age-induced dietary changes, and frailty all independently contribute to the changes in microbiome composition in elderly populations [80]. Copper dyshomeostasis is proposed here not as the sole driver of AD-associated dysbiosis; it is rather an unrecognized factor working synergistically with these other established drivers.

An additional limitation is relying on the pig models for the copper-microbiome evidence. Although pigs are considered a better model relative to rodents due to similar gastrointestinal anatomy, this does not translate to the gut microbiome composition. Direct comparisons have shown that the human gut harbors substantially greater populations of Faecalibacterium relative to pigs [81], and neither Faecalibacterium nor Akkermansia is classified as a core member of the swine gut microbiome despite being a keystone species in the human gut [82]. The specific copper sensitivity thresholds of human-associated strains may therefore differ from their porcine counterparts. Additionally, the copper exposure routes differ between species: pig feeding studies deliver copper directly to the gut lumen at pharmacological doses, whereas in humans, the colonic microbiome is exposed to copper primarily through biliary secretion, which can exceed dietary copper intake and is poorly reabsorbed [73, 83]. These critical differences further underscore the need for proposed in vitro experiments using human-derived bacterial strains and the cross-sectional human cohort study outlined in our testable predictions.

The bidirectional nature of copper dyshomeostasis and the AD progression needs to be addressed. AD pathology itself could potentially alter copper metabolism since amyloid plaques are known to sequester copper in the brain, and neuroinflammation may subsequently affect systemic copper regulation through altered ceruloplasmin processing [84]. If copper elevation were a consequence rather than a cause of AD, the proposed mechanism would be fundamentally inverted. Two lines of evidence argue against this interpretation: Squitti and colleagues demonstrated that baseline serum free copper levels predict future conversion from MCI to AD, establishing that copper elevation precedes clinical disease progression [17]. Additionally, MR analyses provide directionally consistent but methodologically limited genetic evidence linking copper levels to AD risk [18], which should be interpreted alongside the observational evidence rather than treated as independent causal proof. However, once the copper-dysbiosis-neuroinflammation cascade is initiated, bidirectional reinforcement between copper dysregulation and AD pathology likely develops and makes it difficult to disentangle cause from consequence in cross-sectional studies of established disease.

An important complexity to address is the 'copper paradox' in AD, which is the presence of elevated serum free copper while bulk brain copper is decreased, potentially reflecting redistribution into amyloid plaques [84, 85]. This apparent contradiction to our hypothesis can be resolved by recognizing that we specifically refer to elevated non-ceruloplasmin-bound copper in the systemic circulation and gut lumen, not total brain copper. Typically, the gut microbiome is exposed to copper through biliary excretion and mucosal transport, and elevated serum free copper levels have been consistently documented in AD patients [13, 17]. In addition, it is crucial to acknowledge that the prior copper-lowering trials that failed in AD, including the metal protein-attenuating compounds PBT1 and PBT2 [86], targeted brain copper chelation rather than systemic free copper reduction, which may potentially explain their lack of efficacy. Our hypothesis predicts that interventions reducing systemic free copper, rather than brain copper, would ameliorate gut dysbiosis.

Lastly, alternative theories on upstream triggers for AD progression warrant consideration. Min and colleagues proposed the microbial AD (MAD) hypothesis, in which brain-resident microbes trigger antimicrobial responses from Aβ, tau, and ApoE that subsequently deplete neuronal copper and disrupt mitochondrial respiration [87]. In contrast to our gut-centered framework, the MAD hypothesis positions microbial infection as the initial event and focuses on brain copper depletion rather than the peripheral copper elevation. These hypotheses are not mutually exclusive, as copper may contribute to AD simultaneously through both the gut-brain-axis-mediated mechanisms proposed by our hypothesis and through an antimicrobial response in the brain. Similarly, the bile acid hypothesis of AD-related gut dysfunction may converge with rather than compete against our framework, as excess copper has been shown to alter the farnesoid X receptor (FXR) cistrome and impair bile acid signaling in Wilson's disease [88]. Beyond these, APOE4-mediated microbiome shifts, age-related changes in bile acid metabolism, and chronic

neuroinflammation initiating secondary gut dysfunction have each been proposed as upstream drivers of AD-associated dysbiosis independently of copper status. Copper dyshomeostasis is proposed here not as the sole upstream trigger but as one of several age-related factors that may converge synergistically to initiate gut-brain axis dysfunction. The convergence of multiple independent hypotheses on overlapping downstream pathways, including LPS translocation, butyrate depletion, and BBB compromise, reinforces that these mechanisms are likely complementary rather than mutually exclusive.

Conclusions

We have proposed that age-related copper dyshomeostasis serves as a specific, upstream trigger of gut-brain axis dysfunction in AD. The vulnerability of strictly anaerobic butyrate-producing bacteria to copper, which would be maximal under anaerobic conditions, provides a mechanistic explanation for the altered SCFA profile already identified as an AD biomarker. In particular, by disrupting the microbial cross-feeding networks that are necessary for butyrate synthesis, copper-induced depletion of butyrate producers explains the elevated acetate/butyrate ratio that distinguishes AD-related cognitive impairment from non-AD cognitive impairment with remarkable diagnostic accuracy (AUC 0.951). This dysbiosis creates a triple inflammatory insult (Fig. 1) that converges on NF-κB signaling to drive the neuroinflammatory processes underlying AD pathology. Our hypothesis complements rather than competes with Brown's endotoxin hypothesis by providing the missing upstream explanation for why LPS becomes elevated. The therapeutic implications become promising if the hypothesis is confirmed. Unlike downstream targets such as amyloid plaques, copper can be readily measured and modified through existing clinical approaches, and emerging evidence suggests that microbiome modulation can enhance copper-lowering efficacy. We have provided specific, falsifiable predictions that can be tested in human cohorts. The essential next step is an integrated study measuring copper status, microbiome composition, SCFA profiles, LPS, and AD biomarkers in the same aging cohort. Validation of this hypothesis would establish age-related copper accumulation as a modifiable upstream driver of gut-brain axis dysfunction, with implications for neuroinflammation prevention across the aging population.

Competing interests

The author declares that they have no competing interests.

Authors' contributions

AA conceived the hypothesis, conducted the literature review, and wrote the manuscript. The author read and approved the final manuscript.

Acknowledgements

During the preparation of this manuscript, Claude Opus 4.6 (Anthropic) was used to assist with citation verification and manuscript editing. All intellectual content, including the hypothesis, mechanistic framework, and interpretations, was conceived and developed by the author. The author reviewed, revised, and approved all output and takes full responsibility for the content of the published article.

No funding was received for this work.

References

[1] Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. (2019). The Microbiota-Gut-Brain Axis. Physiol Rev, 99:1877–2013.

[2] Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, et al. (2017). Gut microbiome alterations in Alzheimer's disease. Sci Rep, 7:13537.

[3] Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, et al. (2017). Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiol Aging, 49:60–68.

[4] Seo D, Holtzman DM (2024). Current understanding of the Alzheimer's disease-associated microbiome and therapeutic strategies. Exp Mol Med, 56:86–94.

[5] Cammann D, Lu Y, Cummings MJ, Zhang ML, Cue JM, Do J, et al. (2023). Genetic correlations between Alzheimer's disease and gut microbiome genera. Sci Rep, 13:5258.

[6] Brown GC (2019). The endotoxin hypothesis of neurodegeneration. J Neuroinflammation, 16:180.

[7] Marizzoni M, Cattaneo A, Mirabelli P, Festari C, Lopizzo N, Nicolosi V, et al. (2020). Short-Chain Fatty Acids and Lipopolysaccharide as Mediators Between Gut Dysbiosis and Amyloid Pathology in Alzheimer's Disease. J Alzheimers Dis, 78:683–697.

[8] Brown GC, Heneka MT (2024). The endotoxin hypothesis of Alzheimer's disease. Mol Neurodegener, 19:30.

[9] Fu S, Jiang W, Zheng W (2015). Age-dependent increase of brain copper levels and expressions of copper regulatory proteins in the subventricular zone and choroid plexus. Front Mol Neurosci, 8:22.

[10] Peng F, Xie F, Muzik O (2018). Alteration of Copper Fluxes in Brain Aging: A Longitudinal Study in Rodent Using 64CuCl2-PET/CT. Aging Dis, 9:109–118.

[11] Wang W, Lu D, Yang H, Chen Z, Ling W, Song S, et al. (2025). Unveiling the Origin of Copper Accumulation in Plasma with Aging. Environ Health, 3:58–67.
[12] Zhao Z, Lucero MY, Su S, Chaney EJ, Xu JJ, Myszka M, et al. (2025). Activity-based sensing reveals elevated labile copper promotes liver aging via hepatic ALDH1A1 depletion. Nat Commun, 16:1794.
[13] Squitti R, Simonelli I, Ventriglia M, Siotto M, Pasqualetti P, Rembach A, et al. (2013). Meta-Analysis of Serum Non-Ceruloplasmin Copper in Alzheimer's Disease. J Alzheimer's Dis, 38:809–822.
[14] Piacenza F, Giacconi R, Costarelli L, Basso A, Bürkle A, Moreno-Villanueva M, et al. (2021). Age, Sex, and BMI Influence on Copper, Zinc, and Their Major Serum Carrier Proteins in a Large European Population Including Nonagenarian Offspring From MARK-AGE Study. J Gerontol Ser A, 76:2097–2106.
[15] Musci G, Bonaccorsi Di Patti MC, Fagiolo U, Calabrese L (1993). Age-related changes in human ceruloplasmin. Evidence for oxidative modifications. J Biol Chem, 268:13388–13395.
[16] Dupont CL, Grass G, Rensing C (2011). Copper toxicity and the origin of bacterial resistance: new insights and applications. Metallomics, 3:1109.
[17] Squitti R, Ghidoni R, Siotto M, Ventriglia M, Benussi L, Paterlini A, et al. (2014). Value of serum nonceruloplasmin copper for prediction of mild cognitive impairment conversion to Alzheimer disease. Ann Neurol, 75:574–580.
[18] Li Y, Lin H, Liu K, Kan X (2025). Human Trace Elements, Gut Microbiota, and Alzheimer's Disease: Insights From Multistage Mendelian Randomization Analysis. Food Sci Nutr, 13:e70706.
[19] Li J, Yuan Z, Li J, Liu Z, Wang Y, Cui M, et al. (2025). Immune, blood-brain barrier, and metabolic biomarkers mediate gut-brain axis crosstalk in alzheimer's disease. Biomark Res, 13:137.
[20] Marizzoni M, Coppola L, Festari C, Luongo D, Salamone D, Naviglio D, et al. (2025). Circulating short chain fatty acids in Alzheimer's disease: A cross-sectional observational study. J Alzheimer's Dis, 106:38–43.
[21] Bucossi S, Ventriglia M, Panetta V, Salustri C, Pasqualetti P, Mariani S, et al. (2011). Copper in Alzheimer's Disease: A Meta-Analysis of Serum, Plasma, and Cerebrospinal Fluid Studies. J Alzheimers Dis, 24:175–185.
[22] Squitti R, Bressi F, Pasqualetti P, Bonomini C, Ghidoni R, Binetti G, et al. (2009). Longitudinal prognostic value of serum "free" copper in patients with Alzheimer disease. Neurology, 72:50–55.
[23] Squitti R, Pasqualetti P, Dal Forno G, Moffa F, Cassetta E, Lupoi D, et al. (2005). Excess of serum copper not related to ceruloplasmin in Alzheimer disease. Neurology, 64:1040–1046.
[24] Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, et al. (2022). Copper induces cell death by targeting lipoylated TCA cycle proteins. Science, 375:1254–1261.
[25] Cong C, Cong H, Yao Y, Bai Y, Xu L (2025). Copper homeostasis and cuproptosis in Alzheimer's disease (Review). Int J Mol Med, 56:1–21.
[26] Okafor M, Faller P, Vitale N (2025). Cell-specific copper dyshomeostasis mechanism in Alzheimer's disease. Transl Neurodegener, 14:42.
[27] Outten FW, Huffman DL, Hale JA, O'Halloran TV (2001). The Independent cue and cusSystems Confer Copper Tolerance during Aerobic and Anaerobic Growth inEscherichia coli. J Biol Chem, 276:30670–30677.
[28] Lu Z, Imlay JA (2021). When anaerobes encounter oxygen: mechanisms of oxygen toxicity, tolerance and defence. Nat Rev Microbiol, 19:774–785.
[29] Macomber L, Imlay JA (2009). The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proc Natl Acad Sci, 106:8344–8349.
[30] Khan MT, Duncan SH, Stams AJM, Van Dijl JM, Flint HJ, Harmsen HJM (2012). The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic–anoxic interphases. ISME J, 6:1578–1585.
[31] Khan MT, Van Dijl JM, Harmsen HJM (2014). Antioxidants Keep the Potentially Probiotic but Highly Oxygen-Sensitive Human Gut Bacterium Faecalibacterium prausnitzii Alive at Ambient Air. PLoS ONE, 9:e96097.
[32] Vital M, Howe AC, Tiedje JM (2014). Revealing the Bacterial Butyrate Synthesis Pathways by Analyzing (Meta)genomic Data. mBio, 5:e00889-14.
[33] Louis P, Flint HJ (2017). Formation of propionate and butyrate by the human colonic microbiota. Environ Microbiol, 19:29–41.
[34] Wen Y, Yang L, Wang Z, Liu X, Gao M, Zhang Y, et al. (2023). Blocked conversion of Lactobacillus johnsonii derived acetate to butyrate mediates copper-induced epithelial barrier damage in a pig model. Microbiome, 11:218.
[35] Zhao L, Li X, Wang Y, Yang Q, Jiang X, Zhao R, et al. (2024). Resistance role of Lactobacillus sp. and Lactococcus sp. to copper ions in healthy children's intestinal microorganisms. J Hazard Mater, 469:134059.
[36] Fernandez M, Thompson J, Calle A (2024). Novel feed additive delivers antimicrobial copper and influences fecal microbiota in pigs. Microbiol Spectr, 12:e04280-23.
[37] Wen Y, Gao M, Wang Z, Liu X, Zhang Y, Lin G, et al. (2026). Dietary copper-driven colonic dysbiosis mediates oxidative stress and butyrate deficiency to facilitate the spread of resistome in pigs. Npj Biofilms Microbiomes. doi: 10.1038/s41522-026-00949-1.
[38] Zhu Q, Chen B, Zhang F, Zhang B, Guo Y, Pang M, et al. (2024). Toxic and essential metals: metabolic interactions with the gut microbiota and health implications. Front Nutr, 11:1448388.
[39] Shao M, Zhu Y (2020). Long-term metal exposure changes gut microbiota of residents surrounding a mining and smelting area. Sci Rep, 10:4453.
[40] Geng H, Shu S, Dong J, Li H, Xu C, Han Y, et al. (2018). Association study of gut flora in Wilson's disease

through high-throughput sequencing. Medicine (Baltimore), 97:e11743.

[41] Cai X, Deng L, Ma X, Guo Y, Feng Z, Liu M, et al. (2020). Altered diversity and composition of gut microbiota in Wilson's disease. Sci Rep, 10:21825.

[42] Cai X, Dai J, Xie Y, Xu S, Liu M (2024). Multi-omics study unravels gut microbiota and metabolites alteration in patients with Wilson's disease. Sci Rep, 14:21025.

[43] Wei T, Qian N, Wang H, Song Y, Wang W, Li Y, et al. (2026). Wilson's disease-associated gut dysbiosis: novel insights into microbial functional alterations, virulence changes, and resistance markers. Front Microbiol, 16:1714276.

[44] Zhong H, Liu A, Huang D, Zhou Z, Xu S, Wu L, et al. (2024). Exploring the impact of gut microbiota on liver health in mice and patients with Wilson disease. Liver Int, 44:2700–2713.

[45] Sarode GV, Mazi TA, Neier K, Shibata NM, Jospin G, Harder NHO, et al. (2023). The role of the intestine in metabolic dysregulation in murine Wilson disease. Hepatol Commun. doi: 10.1097/HC9.0000000000000247.

[46] Liu J, Zhang Z, Zhong S, Zhang X, Yang J, Zhou Q, et al. (2024). Fecal microbiome transplantation alleviates manganese-induced neurotoxicity by altering the composition and function of the gut microbiota via the cGAS–STING/NLRP3 pathway. Sci Total Environ, 951:175681.

[47] Kania B, Sotelo A, Ty D, Wisco JJ (2023). The Prevention of Inflammation and the Maintenance of Iron and Hepcidin Homeostasis in the Gut, Liver, and Brain Pathologies. J Alzheimer's Dis, 92:769–789.

[48] Miller KA, Vicentini FA, Hirota SA, Sharkey KA, Wieser ME (2019). Antibiotic treatment affects the expression levels of copper transporters and the isotopic composition of copper in the colon of mice. Proc Natl Acad Sci, 116:5955–5960.

[49] Huang X, Jin Y, Wang T, Fu D, Ma J, Yu X, et al. (2025). Gut *Akkerm*ansia enhances liver protection and facilitates copper removal during D-penicillamine treatment in a Wilson's disease model. Microbiol Spectr, 13:e00573-24.

[50] Feng P, Yang J, Zhao S, Ling Z, Han R, Wu Y, et al. (2022). Human supplementation with Pediococcus acidilactici GR-1 decreases heavy metals levels through modifying the gut microbiota and metabolome. Npj Biofilms Microbiomes, 8:63.

[51] Hodgkinson K, El Abbar F, Dobranowski P, Manoogian J, Butcher J, Figeys D, et al. (2023). Butyrate's role in human health and the current progress towards its clinical application to treat gastrointestinal disease. Clin Nutr, 42:61–75.

[52] Romo EZ, Hong BV, Patel RY, Agus JK, Harvey DJ, Maezawa I, et al. (2024). Elevated lipopolysaccharide binding protein in Alzheimer's disease patients with APOE3/E3 but not APOE3/E4 genotype. Front Neurol, 15:1408220.

[53] Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, et al. (2016). Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. Cell, 165:842–853.

[54] d'Hennezel E, Abubucker S, Murphy LO, Cullen TW (2017). Total Lipopolysaccharide from the Human Gut Microbiome Silences Toll-Like Receptor Signaling. mSystems, 2:e00046-17.

[55] Giangiulio O, Maccarone R (2025). The Blood–Brain Barrier as an Integration Hub in Alzheimer's Disease: How Microbiota Metabolites Modulate Central Signal Processing. CNS Neurosci Ther, 31:e70703.

[56] Veerareddy V, Wang Z, Kashyap PC, Kandimalla KK (2025). Butyrate regulates the blood-brain barrier transport and intra-endothelial accumulation of Alzheimer's disease Amyloid-beta peptides. bioRxiv. doi: 10.1101/2025.10.24.684335.

[57] Wei C, Jiang W, Wang R, Zhong H, He H, Gao X, et al. (2024). Brain endothelial GSDMD activation mediates inflammatory BBB breakdown. Nature, 629:893–900.

[58] Li Y-Q, Tan S-S, Wu D, Zhang Q, Wang T, Zheng G (2025). The role of intracellular and extracellular copper compartmentalization in Alzheimer's disease pathology and its implications for diagnosis and therapy. Front Neurosci, 19:1553064.

[59] Parada-Venegas D, De La Fuente López M, Dubois-Camacho K, Landskron G, Blokzijl T, Molina H, et al. (2025). Butyrate suppresses mucosal inflammation in inflammatory bowel disease primarily through HDAC3 inhibition in monocytes and macrophages. FEBS J, 292:6134–6157.

[60] Erny D, Hrabě De Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci, 18:965–977.

[61] Mishra SP, Jain S, Wang B, Wang S, Miller BC, Lee JY, et al. (2024). Abnormalities in microbiota/butyrate/FFAR3 signaling in aging gut impair brain function. JCI Insight, 9:e168443.

[62] Sun J, Lu L, Lian Y, Xu S, Zhu Y, Wu Y, et al. (2025). Sodium butyrate attenuates microglia-mediated neuroinflammation by modulating the TLR4/MyD88/NF-κB pathway and microbiome-gut-brain axis in cardiac arrest mice. Mol Brain, 18:13.

[63] Tao F, Lin M, Meng X, Huang L, Zhuo B, Jiang S, et al. (2025). Copper homeostasis and cuproptosis: implications for neurodegenerative diseases. Front Aging Neurosci, 17:1688554.

[64] Colombo AV, Sadler RK, Llovera G, Singh V, Roth S, Heindl S, et al. (2021). Microbiota-derived short chain fatty acids modulate microglia and promote Aβ plaque deposition. eLife, 10:e59826.

[65] Seo D, O'Donnell D, Jain N, Ulrich JD, Herz J, Li Y, et al. (2023). ApoE isoform– and microbiota-dependent progression of neurodegeneration in a mouse model of tauopathy. Science, 379:eadd1236.

[66] Akhgarjand C, Vahabi Z, Shab-Bidar S, Etesam F, Djafarian K (2022). Effects of probiotic supplements on cognition, anxiety, and physical activity in subjects with mild and moderate Alzheimer's disease: A randomized, double-blind, and placebo-controlled study. Front Aging Neurosci, 14:1032494.

[67] Rashid F, Khan KM, Saiprakash S, Ahmed G, Sultana R, Parvez F, et al. (2025). Epidemiological Evidence on the Associations of Metal Exposure with Alzheimer's Disease and Related Dementias Among Elderly Women. J Clin Med, 14:3776.

[68] Cummings J, Zhou Y, Lee G, Zhong K, Fonseca J, Cheng F (2024). Alzheimer's disease drug development pipeline: 2024. Alzheimers Dement Transl Res Clin Interv, 10:e12465.

[69] Zhu X, Victor TW, Ambi A, Sullivan JK, Hatfield J, Xu F, et al. (2020). Copper accumulation and the effect of chelation treatment on cerebral amyloid angiopathy compared to parenchymal amyloid plaques. Metallomics, 12:539–546.

[70] Ambi A, Stanisavljevic A, Victor TW, Lowery AW, Davis J, Van Nostrand WE, et al. (2023). Evaluation of Copper Chelation Therapy in a Transgenic Rat Model of Cerebral Amyloid Angiopathy. ACS Chem Neurosci, 14:378–388.

[71] Camargo MLM, Farias AB, Bertazzo GB, Gomes RN, Gomes KS, Bosquetti LM, et al. (2025). Novel Copper Chelators Enhance Spatial Memory and Biochemical Outcomes in Alzheimer's Disease Model. ACS Chem Neurosci, 16:3267–3281.

[72] Agahi A, Hamidi GA, Daneshvar R, Hamdieh M, Soheili M, Alinaghipour A, et al. (2018). Does Severity of Alzheimer's Disease Contribute to Its Responsiveness to Modifying Gut Microbiota? A Double Blind Clinical Trial. Front Neurol, 9:662.

[73] European Association for the Study of the Liver (2012). EASL Clinical Practice Guidelines: Wilson's disease. J Hepatol, 56:671–685.

[74] Forouzandeh A, Lassen SB, Brinck JE, Zhou YY, Zhu J, Solà-Oriol D, et al. (2023). Limited impacts of high doses of dietary copper on the gut bacterial metal resistome explain negligible co-selection of antibiotic resistance. Sci Total Environ, 889:164183.

[75] Brinck JE, Lassen SB, Forouzandeh A, Pan T, Wang Y-Z, Monteiro A, et al. (2023). Impacts of dietary copper on the swine gut microbiome and antibiotic resistome. Sci Total Environ, 857:159609.

[76] Chen H, Li D, Zhang H, Zhang M, Lin Y, He H, et al. (2025). Mechanisms of copper metabolism and cuproptosis: implications for liver diseases. Front Immunol, 16:1633711.

[77] Conway J, De Jong EN, White AJ, Dugan B, Rees NP, Parnell SM, et al. (2025). Age-related loss of intestinal barrier integrity plays an integral role in thymic involution and T cell ageing. Aging Cell, 24:e14401.

[78] Meng L, Wang Z, Ming Y-C, Shen L, Ji H-F (2022). Are micronutrient levels and supplements causally associated with the risk of Alzheimer's disease? A two-sample Mendelian randomization analysis. Food Funct, 13:6665–6673.

[79] Evans DM, Zhu G, Dy V, Heath AC, Madden PAF, Kemp JP, et al. (2013). Genome-wide association study identifies loci affecting blood copper, selenium and zinc. Hum Mol Genet, 22:3998–4006.

[80] Haran JP, Zeamer A, Ward DV, Dutta P, Bucci V, McCormick BA (2021). The Nursing Home Older Adult Gut Microbiome Composition Shows Time-dependent Dysbiosis and Is Influenced by Medication Exposures, Age, Environment, and Frailty. J Gerontol Ser A, 76:1930–1938.

[81] Xiang Z, Zhu H, Yang B, Fan H, Guo J, Liu J, et al. (2020). A glance at the gut microbiota of five experimental animal species through fecal samples. Sci Rep, 10:16628.

[82] Holman DB, Brunelle BW, Trachsel J, Allen HK (2017). Meta-analysis To Define a Core Microbiota in the Swine Gut. mSystems, 2:e00004-17.

[83] Linder MC (2020). Copper Homeostasis in Mammals, with Emphasis on Secretion and Excretion. A Review. Int J Mol Sci, 21:4932.

[84] Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci, 158:47–52.

[85] Xu J, Church SJ, Patassini S, Begley P, Waldvogel HJ, Curtis MA, et al. (2017). Evidence for widespread, severe brain copper deficiency in Alzheimer's dementia. Metallomics, 9:1106–1119.

[86] Sampson EL, Jenagaratnam L, McShane R (2014). Metal protein attenuating compounds for the treatment of Alzheimer's dementia. Cochrane Database Syst Rev. doi: 10.1002/14651858.CD005380.pub5.

[87] Min J-H, Sarlus H, Harris RA (2024). MAD—microbial (origin of) Alzheimer's disease hypothesis: from infection and the antimicrobial response to disruption of key copper-based systems. Front Neurosci, 18:1467333.

[88] Wooton-Kee CR, Yalamanchili HK, Mohamed I, Hassan M, Setchell KDR, Narvaez Rivas M, et al. (2025). Changes in the FXR-cistrome and alterations in bile acid physiology in Wilson disease. Hepatol Commun. doi: 10.1097/HC9.0000000000000707.