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Review

Metformin, Cognitive Function, and Changes in the Gut Microbiome

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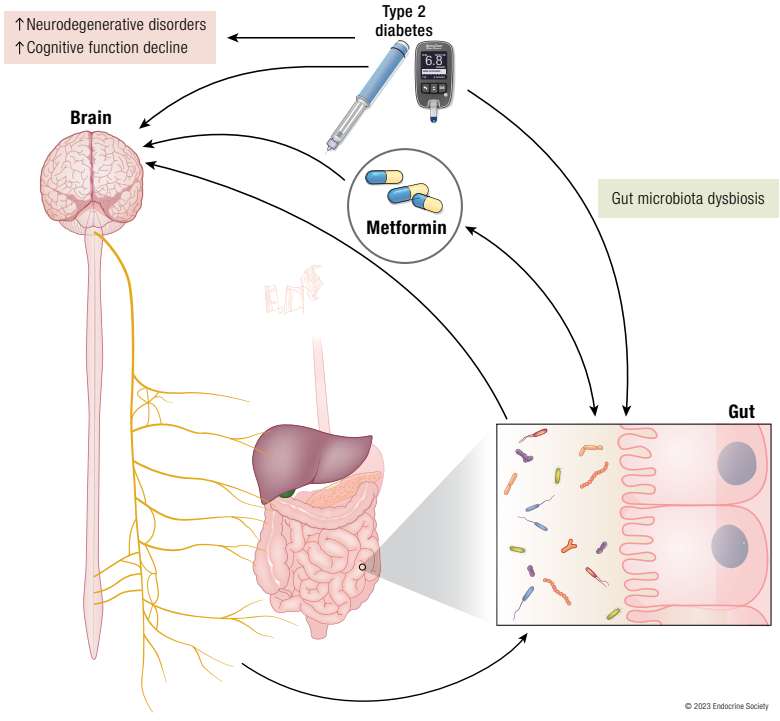
Abstract

The decline in cognitive function and the prevalence of neurodegenerative disorders are among the most serious threats to health in old age. The prevalence of dementia has reached 50 million people worldwide and has become a major public health problem. The causes of age-related cognitive impairment are multiple, complex, and difficult to determine. However, type 2 diabetes (T2D) is linked to an enhanced risk of cognitive impairment and dementia. Human studies have shown that patients with T2D exhibit dysbiosis of the gut microbiota. This dysbiosis may contribute to the development of insulin resistance and increased plasma lipopolysaccharide concentrations. Metformin medication mimics some of the benefits of calorie restriction and physical activity, such as greater insulin sensitivity and decreased cholesterol levels, and hence may also have a positive impact on aging in humans. According to recent human investigations, metformin might partially restore gut dysbiosis related to T2D. Likewise, some studies showed that metformin reduced the risk of dementia and improved cognition, although not all studies are concordant. Therefore, this review focused on those human studies describing the effects of metformin on the gut microbiome (specifically the changes in taxonomy, function, and circulating metabolomics), the changes in cognitive function, and their possible bidirectional implications.

Graphical Abstract



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Key Words: metformin, type 2 diabetes, gut microbiota, cognition

Abbreviations: AMPK, adenosine monophosphate–activated protein kinase; GLP-1, glucagon-like peptide; GUDCA, glycoursodeoxycholic acid; hNSC, human neural stem cell; HSDH, hydroxysteroid dehydrogenase; SCFA, short-chain fatty acid; T2D, type 2 diabetes.

ESSENTIAL POINTS

- Metformin seems to exert neuroprotective, anti-inflammatory, and antioxidant effects, including prevention of dementia and neurodegenerative diseases in humans
- Clinical studies have also reported an association between metformin and improved memory and executive function
- Metformin use changes bacterial taxonomy, increasing the relative abundance of species such as A*kkermansia muciniphila* and *Escherichia coli*
- Likewise, bacterial functionality changes after metformin, increasing different short-chain fatty acids and bile acids
- Although research on the gut microbiota-gut–brain–metformin axis has reported interesting and consistent associations in some human studies, most studies are purely associative
- Understanding the complex metformin–gut–brain axis interactions could allow a personalized medicine, targeting the beneficial actions of metformin as well as diminish its adverse effects

Metformin Cognition–Gut Microbiota

The decline in cognitive function and the prevalence of neurodegenerative disorders are among the most serious threats to health in old age. Mild cognitive impairment occurs in about 40% of the population aged ≥65 years and is associated with a lower quality of life and increased incidence of associated pathology (1). The prevalence of dementia has reached 50 million people worldwide and has become a major public health problem. Around 2050, this number will increase 3-fold (2). The causes of age-related cognitive impairment are multiple, complex, and difficult to determine. However, the risk of developing inflammation and cognitive impairment increases in the presence of metabolic diseases such as type 2 diabetes (T2D) and hypercholesterolemia. Low-grade systemic inflammation associated with metabolic disorders has been reported to affect the brain as people age (3).

The microbiome-gut–brain axis is a bidirectional communication system. This complex system involves the gut microbiota, the enteric nervous system, the autonomic nervous system, the neuroendocrine system, and the central nervous system. This relationship is facilitated by the vagus nerve, the circulatory system, and the immune system (4). The influence of the gut microbiota on the development of the gut–brain axis is crucial at birth. A microbiome categorized as "pathogenic" can independently initiate disease even in individuals who do not possess inherent susceptibility (5). Modifying the microbiome through dietary changes and other interventions could alter its function, and whether it occurs within an individual or is inherited by future generations (6). Therefore, phenotypes in response to a dysbiosis of the gut microbiota could be expected in the next generation. This was demonstrated in mice, where fecal microbial transplantation from patients with autism spectrum disorder was sufficient to promote autism-like behavioral changes in the offspring (7).

The gut microbiota can influence a wide range of physiological processes. Furthermore, it has been identified as an important factor in modulating brain function and structure. In this sense, fecal microbiota composition has been found to be associated with cognition in humans. For instance, bacterial species from the Bacteroidetes (*Bacteroides fragilis CAG:558*, *Bacteroides caccae CAG:21*) and the Proteobacteria phyla (*Citrobacter freundii*, *Enterobacter cloacae*, *Salmonella enterica*, and *Klebsiella aerogenes*) were negatively associated with immediate memory scores (8). Conversely, species of the Firmicutes phylum (*Clostridium* sp. *27_14 or Clostridium* sp. *CAG:230*) were positively associated with better scores in tests measuring executive function (working memory) and verbal and learning memory (8). On the other hand, species belonging to the Bacteroidetes phylum (*Bacteroides plebeius*, *Bacteroides gallinarum*, *Bacteroides mediterranensis*) have been negatively associated with inhibitory control (executive function), whereas positive associations with executive performance were found with *Eubacterium* sp. *CAG:603* and *Firmicutes bacterium CAG:238* (8, 9). In addition, the fecal microbiota is involved in the development and progression of various psychiatric and neurological conditions, such as depression, autism, stroke, Parkinson disease, and Alzheimer disease (10).

The biguanide metformin is a widely used drug for the treatment of T2D (11-14). Metformin lowers glucose levels by suppressing hepatic gluconeogenesis. It is widely accepted that the pleiotropic effects of the drug are due to its action on the mitochondria causing a mild and specific inhibition of the respiratory chain complex 1. Inhibition of this complex leads to a decrease in cellular energy charge. The resulting drop in cellular energy activates adenosine monophosphate–activated protein kinase (AMPK). Once activated, AMPK activates the catabolic pathways that generate ATP and deactivates those cellular processes that consume ATP. Although mitochondria play an important role in the mechanism of action of biguanides, not all their effects are mediated by this organelle. It has been proposed that metformin may also activate AMPK through a mechanism involving a lysosomal protein (mTOR activator 1) (12-14).

However, not all therapeutic effects of metformin are mediated by AMPK. Metformin could lead to inhibition (via AMP) of fructose-1,6-bisphosphatase, a key enzyme in gluconeogenesis (12-14). Additionally, metformin treatment increases glucagon-like peptide 1 (GLP-1) concentrations, which appears to be another pathway by which it impacts glucose homeostasis (12-14). GLP-1 is a hormone synthesized and secreted by the brain and the L cells in the intestine in response to nutrients and bacterial factors (15). In diabetic rats with cerebral ischemic damage, recombinant GLP-1 reduced the neurological deficit and infarct area by inhibiting oxidative stress and apoptosis. Treatment with SLAB51 (formulation

of 9 live bacterial strains: *Streptococcus thermophilus*, *B. longum*, *B. breve*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. delbrueckii*, and *L. brevis*) increased plasma GLP-1 concentrations, leading to a reduction in brain amyloid load and resulting in modulation of neuronal functions such as learning and memory in Alzheimer disease mice models (15). Compared with T2D patients on a low-fat diet, T2D patients on a low-carbohydrate almond-based diet had higher GLP-1 levels and an increased relative abundance of bacteria that produce short-chain fatty acids (SCFAs), including *Roseburia*, *Ruminococcus*, and *Eubacterium*. At the conclusion of the trial, this group of patients with T2D displayed improved glycemic control and a lower depression test score (16) Although the results are promising, the role of GLP-1 is not as thoroughly studied and characterized in humans as in animal models.

On the other hand, human studies have shown that patients with T2D exhibit dysbiosis of the fecal microbiota (17, 18). This dysbiosis may contribute to the development of insulin resistance and increased plasma lipopolysaccharide concentrations (19). According to recent human investigations, metformin might partially restore gut dysbiosis related to T2D (20-22). Metformin medication mimics some of the benefits of calorie restriction and physical activity, such as greater insulin sensitivity (23) and decreased cholesterol levels (24), and hence may also have a positive impact on aging in humans (25, 26). Besides, some studies showed that metformin reduced the risk of dementia (27, 28). We aimed to review here those studies related to metformin and its relationship with the gut microbiome and cognition in humans.

Impact of Metformin on Cognition

T2D is related to an enhanced risk of cognitive impairment and dementia (29). In fact, both insulin resistance and increased circulating glucose levels are associated with poor attention and executive function (processing speed, sustained attention, and working memory) (30) and memory processes (31, 32). Although the mechanisms by which metformin has neuroprotective effects are not fully elucidated, metformin primarily exerts its effects on energy homeostasis within neurons by targeting AMPK (33). In a study involving human neural stem cells (hNSCs), it was observed that cells treated with amyloid-beta exhibited a substantial decrease in cell viability. This reduction was found to be associated with lowered expressions of AMPK, neuroprotective genes (Bcl-2 and CREB), and mitochondria-associated genes (PGC1α, NRF-1, and Tfam), while also showing heightened activation of caspase 3/9 and increased cytosolic cytochrome c activity (33). Metformin treatment effectively eliminated the detrimental effects induced by amyloid-beta in hNSCs through downregulation of caspase 3/9 activities and cytosolic cytochrome c. Furthermore, metformin cotreatment was critical in restoring the fragmented mitochondria in amyloid-beta–affected hNSCs, approaching normal morphology (33). Besides, metformin has been described to alleviate Alzheimer disease–associated neuropathological changes in differentiated mouse neuroblastoma cell line Neuro-2a (34) and to reduce tau protein phosphorylation in cultured neurons and mouse brains (35). Metformin has also been demonstrated to protect against apoptotic cell death in primary cortical neurons (36). It can normalize the reduced cell proliferation and neuroblast differentiation induced by T2D in the dentate gyrus of the rat hippocampus (37). Lastly, metformin-induced AMPK activation protected hNSCs against cytotoxicity induced by advanced glycation end products (38).

Regarding cognition, the cognitive impairment induced by scopolamine was reversed by 100 mg/kg/day of metformin in male Wistar rats. Metformin demonstrated a reduction in impairments related to spatial learning and memory and short-term working memory. This beneficial effect was linked to a decrease in inflammation and oxidative stress via Akt activation (39). Other research examined the effects of chronic metformin treatment and a chronic high-fat diet on middle-aged male (12 months) C57BL/6 mice. The findings indicated enhanced spatial learning abilities, improved coordination during running tasks, and a decrease in memory impairments (40). However, this study had some limitations, such as the absence of a control group receiving metformin treatment. Finally, in male Wistar rats, the administration of metformin at a dosage of 100 mg/kg/day reversed the cognitive deterioration linked to both a high-fat diet and chronic restraint stress (41). These 2 studies consider latency as the parameter used for cognitive assessment in the Morris water maze testing. Escape latency can be affected by factors such as elevated body weight, diminished motor function, or slower swimming speeds. The body weight of these rats was influenced by the high-fat diet, metformin treatment, and chronic restraint stress, indicating that latency may not provide an accurate estimate for cognition.

Over the last decade, interest has grown in the possible effects of metformin on cognition in human subjects. Several retrospective and prospective studies have been conducted to elucidate whether metformin exerts (or not) benefits on cognition (Fig. 1), as summarized below.

Positive Effects of Metformin on Cognition

Metformin treatment has been linked to a significantly lower risk of dementia (27, 28, 42, 43) and neurodegenerative diseases (44, 45) and with improvement in 3 cognitive domains, memory, semantic memory, and executive function (30, 46-51), mostly in subjects with T2D (Table 1).

One of the most populated studies evaluated Australian individuals aged 70-90 years over the course of 6 years (n = 103 767 with T2D and metformin). Ninety-one cases of dementia during the 6-year study were reported, of which 73 were subjects without T2D (8.2% of that group), 8 were T2D without metformin (14.5% of that group), and 4 were T2D with metformin (6% of that group). In T2D subjects without metformin, the rate of decline was accelerated specifically in executive function ($P = .006$). Hence, an 81% reduction in incident dementia risk was reported in T2D subjects with metformin treatment (HR 0.19, 95% CI 0.04-0.85; $P = .030$) (42).

Consistently, an observational study included 559 106 US veterans over 60 years of age with a diagnosis of T2D. The incidence rate of dementia was lower in the group of patients treated with metformin (6.2 cases per 1000 person-years) than the groups treated with sulfonylureas and thiazolidinedione (13.4 cases per 1000 person-years). Combination therapy with metformin and thiazolidinedione reduced the risk of all-cause dementia. After 2 years of treatment, the combination of metformin and sulfonylureas became protective against all causes of dementia (HR 0.91, 95% CI 0.88-0.95) (43).

Table 1. Human studies focused on metformin and cognition

Reference and Clinical Trials.gov identifier	Country	Study design	Findings related to cognition
(51) (NCT02040376)	Canada	24 surviving pediatric brain tumor subjects.	In the metformin-treated group compared with the placebo-treated group: greater total numbers of correct responses on List Sorting Working Memory ($P = .05$) were observed. Also, a higher information processing speed was noted (decreased average latency on the Cambridge Neuropsychological Test Automated Battery) ($P = .03$). The highest total number of words recalled for immediate recall on the Children's Auditory Verbal Learning Test-2/Rey Auditory Verbal Learning Test ($P = .01$) and a significant increase in the axonal water fraction within the corpus callosum ($P = .04$) were observed.
(52)	United States	508 T2D subjects.	There was no significant association between metformin and cognitive performance on cognitive tests. Metformin was associated with an enhanced risk of mild cognitive impairment (subhazard ratio = 2.75; 95% CI 1.64-4.63, $P < .001$).
(42)	Australia	67 T2D patients with metformin (mean age 78.25 ± 4.6), 56 T2D patients without metformin (mean age 80.0 ± 4.7), 903 nondiabetic individuals (mean age 78.8 ± 4.8).	No differences in baseline cognitive performance were found between the groups. In T2D subjects with metformin, a significantly lower rate of decline over 6 years was reported ($P = .032$). In addition, in T2D subjects without metformin, the rate of decline was accelerated for executive function ($P = .006$), memory, language, and attention/processing speed (without reaching statistical significance). Hence, an 81% reduction in incident dementia risk was reported in T2D subjects with metformin treatment (HR 0.19, 95% CI 0.04-0.85, $P = .030$)
(53)	South Korea	93 T2D patients with metformin (mean age 75.8 ± 6.5), 64 T2D patients without metformin (mean age 77.2 ± 7.4), 575 nondiabetic individuals (mean age 76.8 ± 6.7).	Metformin was related with fast decline of Mini-Mental State Examination scores (OR 4.47, 95% CI 1.24-16.05, $P = .022$) and Verbal Immediate Recall scores (OR 7.37, 95% CI 1.19-45.56, $P = .032$).
(49)	Australia	74 T2D patients with diet control (mean age 66.3 ± 1.5), 24 T2D patients with insulin treatment (mean age 67.3 ± 0.3), 113 T2D patients with oral hypoglycemic agents (mean age 66.7 ± 1.6), 1603 nondiabetic individuals (mean age 66.6 ± 1.5).	In 23 patients with T2D on metformin-only treatment, significantly better performance was observed on the Immediate Recall (verbal memory, $P < .001$), Digit Span Backward (working memory, $P < .01$), and Trail Making Test Part B (executive function, $P < .01$) tests at baseline. It was reported in the longitudinal analysis that participants with exclusive metformin (n = 76) had a significant protective effect on performance for choice reaction time ($P < .01$).
(48)	Canada	1192 subjects with normal cognition (mean age 72.25 ± 8.28), 671 subjects with mild cognitive impairment (mean age 74.37 ± 8.2), 807 subjects with AD dementia (mean age 76.11 ± 7.93).	In patients with T2D who received metformin-only treatment, improvements in the performance of immediate memory (β = .069, 95% CI 0.01-0.12, $P = .0202$) and delayed memory (β = .089, 95% CI 0.032-0.146, $P = .0024$) was observed across time. Metformin did not display a significant association with memory changes in patients with mild cognitive impairment and AD dementia.
(30)	United States	2925 participants.	In the metformin-treated group compared with subjects with other anti-diabetic drugs (n = 311): significantly higher scores on DSST and AFT were found; hence, increased linguistic fluency as assessed with AFT, and enhanced executive function, as assessed by the DSST.
(44)	United States	6046 patients with T2D (mean age 63.20 ± 10.90 years), 2993 without metformin treatment (mean age 65.82 ± 11.66 years), 932 patients with metformin treatment ≤1 year, (mean age 61.25 ± 10.74 years), 566 with metformin treatment	In metformin-treated group compared with no treated group: ≤1 year of metformin treatment was associated with an increased risk of neurodegenerative disease (HR 1.16, 95% CI 0.89-1.51).

(*continued*)

Table 1. Continued

Reference and Clinical Trials.gov identifier	Country	Study design	Findings related to cognition
		between 1-2 years (mean age 60.68 ± 9.16 years), 789 patients with metformin treatment between 2 and 4 years (mean age 60.23 ± 8.98 years), 766 patients with metformin treatment > 4 years (mean age 60.21 ± 8.24 years).	1-2 years of metformin therapy reduced neurodegenerative disease risk without statistical significance (HR 0.80, 95% CI 0.56-1.13). 2-4 years (HR 0.62, 95% CI 0.45-0.85) and ≥4 years (HR 0.19, 95% CI 0.12-0.31) of metformin use were linked to a significative lower risk of neurodegenerative disease.
(50) (NCT01965756)	US	20 subjects (mean age 70.1 ± 6.89 years)	Metformin was found to be associated with enhanced executive functioning (TMT-B: $P = .03$), and trends suggested improvement in learning/memory (Paired Associates Learning: $P = .06$) and attention (Delayed Matching to Sample: $P = .07$). In addition, during the 8 weeks of metformin treatment, was found a significant increase in superior and middle orbitofrontal cerebral blood flow ($P < .05$).
(27)	US	17 200 subjects using metformin, 11 440 subjects using sulfonylureas (mean age 73.5 ± 5.9 years)	In metformin users compared to sulfonylurea users was noted a lower risk of dementia in patients <75 years old (HR 0.67, 95% CI 0.61-0.73, $P < .001$). The same result was obtained in patients ≥ 75 years (HR 0.78, 95% CI 0.72-0.83, $P < .001$). After adjusting for the IPTW method, metformin remained protective against the risk of dementia but only in <75 years old patients (HR 0.89; 95% CI 0.79-0.99, $P < .033$).
(28)	Taiwan	An unmatched cohort of 147 729 metformin-treated patients and 15 676 nontreated subjects. Another matched cohort of 15 676 people with metformin and 15 676 people without metformin.	Metformin consumption was related to a lower risk of dementia in the unmatched cohort (HR 0.550, 95% CI 0.508-0.596, $P < .0001$) and in the matched cohort (HR 0.707, 95% CI 0.632-0.791, $P < .0001$).
(46) (NCT00620191)	US	40 subjects taking metformin (mean age 65.3 ± 7.0 years), 40 subjects taking placebo (mean age 64.1 ± 7.9 years).	In the metformin-treated group compared with the placebo-treated group: In crude analysis, higher improvement in SRT was found. However, after controlling for baseline ADAS-Cog, the metformin group showed a considerable improvement in SRT total recall ($P = .02$). The highest dose of metformin was correlated to a statistically significant increase in total SRT recall words.
(47)	Spain	148 subjects with T2D (mean age 65.9 ± 4.7 years), 339 nondiabetic individuals (mean age 64.9 ± 4.7 years).	At baseline, subjects taking metformin had better performance in executive functions ($d = 0.51$, 95% CI −0.06 to 1.08; $P = .086$), memory ($d = 0.38$, 95% CI −0.02 to 0.79; $P = .115$), and global cognition ($d = 0.48$, 95% CI −0.01 to 1.04; $P = .124$). In contrast, those without metformin had a better performance in executive functions (mean change of 0.36 vs 0.02, $P = .005$), and global cognition (mean change of 0.29 vs −0.02, $P = .001$) after 3 years. Nondiabetic individuals exhibited greater increases in memory ($P < .001$) and global cognition ($P = 003$) after 1 and 3 years compared with subjects on T2D with metformin. T2D subjects without metformin exhibited a better mean rate of change in executive functions compared with nondiabetic individuals ($P = .032$).
(54)	US	2280 participants (749 with lifestyle intervention, 776 with metformin intervention, 755 with placebo intervention) (mean age 63.1 ± 10.7 years).	There were no cognitive differences between the intervention groups. Metformin exposure over time had no effect on cognition.
(55)	US	333 breast cancer survivors (mean age 62.6 ± 6.9 years).	In 5 neurocognitive domains, there were no statistically significant intervention effects for metformin or weight loss.

(*continued*)

Table 1. Continued

Reference and Clinical Trials.gov identifier	Country	Study design	Findings related to cognition
(45)	China	527 138 middle-aged Europeans (24 087 subjects with AD, 47 793 subjects with AD by proxy, and 383 378 controls).	In nondiabetics, genetically proxied metformin use was linked to a 4% decreased risk of AD disease (OR 0.96, 95% CI 0.95-0.98, $P = 1.06\times10^{-4}$). Mitochondrial complex 1 demonstrated a significant impact on AD that was independent of AMPK (OR 0.88, $P = 4.73\times10^{-4}$). A lower risk of AD was linked to reduced mitochondrial complex 1-related gene (NDUFA2) expression (OR 0.95, $P = 4.64\times10^{-4}$).
(43)	US	559 106 subjects with T2D (mean age 65.7 ± SD 8.7)	The incidence rate of dementia was lower in the group of patients treated with metformin (6.2 cases per 1000 person-years) compared with the groups treated with sulfonylureas and thiazolidinedione (13.4 cases per 1000 person-years). Sulfonylurea monotherapy was associated with a 12% higher risk of all causes of dementia compared to the metformin-treated group (HR 1.12, 95% CI 1.09-1.15). Combination therapy with metformin and thiazolidinedione reduced the risk of all-cause dementia. After 2 years of treatment, these results did not change, although the combination of metformin and sulfonylureas became protective against all causes of dementia (HR 0.91, 95% CI 0.88-0.95).

Abbreviations: AD, Alzheimer disease; ADA-Cog, Disease Assessment Scale-cognitive subscale; AFT, Animal Fluency Test; AMPK, adenosine monophosphate–activated protein kinase; DSST, Digit Symbol Substitution Test; IPTW, inverse probability of treatment weighting; OR, odds ratio; SRT, Selective Reminding Test; T2D, type 2 diabetes.

Likewise, an unmatched cohort (n = 147 729 subjects who used metformin) and a matched cohort (n = 15 676 subjects who used metformin and n = 15 676 subjects who never took metformin) were investigated in Taiwan. This study informed a lower risk of dementia in patients taking metformin in both the unmatched cohort (HR 0.550, 95% CI 0.508-0.596, $P < .0001$) and the matched cohort (HR 0.707, 95% CI 0.632-0.791, $P < .0001$) (28).

A cohort study enrolled 17 200 subjects over 65 years of age from the United States with a medical history of T2D. The investigation showed a lower risk of dementia in patients <75 years old in metformin users than in sulfonylurea users (HR 0.67, 95% CI 0.61-0.73, $P < .001$). The same result was noted in patients using metformin ≥75 years (HR 0.78, 95% CI 0.72-0.83, $P < .001$). After adjusting for confounding factors, metformin remained protective against the risk of dementia but only in patients <75 years (HR 0.89, 95% CI 0.79-0.99, $P < .033$) (27).

A longitudinal cohort study examined 6046 patients from the United States with T2D (n = 2993 without metformin, n = 932 patients with metformin ≤1 year, n = 566 with metformin between 1 and 2 years, n = 789 patients with metformin between 2 and 4 years, n = 766 patients with metformin > 4 years). The results revealed that the use of metformin for 2 to 4 years (HR 0.62, 95% CI 0.45-0.85) and over 4 years (HR 0.19, 95% CI 0.12-0.31) was associated with a significant reduction in the incidence of neurodegenerative disease (dementia, Parkinson disease, Alzheimer disease, cognitive impairment). In general, the results for each neurodegenerative disease subtype resembled the results obtained with the neurodegenerative disease (44).

In another report, 527 138 middle-aged Europeans were evaluated (24 087 subjects with Alzheimer disease, 47 793 subjects with Alzheimer disease by proxy, and 383 378 control subjects). These authors used Mendelian randomization to investigate the effect and mechanisms of metformin on Alzheimer disease. Mitochondrial complex 1 demonstrated a significant impact on Alzheimer disease (odds ratio [OR] 0.88, $P = 4.73\times10^{-4}$). A lower risk of Alzheimer disease was linked to reduced Mitochondrial complex 1-related (NDUFA2) gene expression (OR 0.95, $P = 4.64\times10^{-4}$). In the nondiabetic population, genetically proxied metformin use was linked to a 4% decreased risk of Alzheimer disease (OR 0.96, 95% CI 0.95-0.98, $P = 1.06\times10^{-4}$) (45).

Regarding to cognition, a survey in Canada included T2D subjects (1192 subjects with normal cognition, 671 subjects with mild cognitive impairment, and 807 subjects with AD dementia). Metformin treatment improved immediate memory (standardized coefficients (β) = .069, 95% CI 0.01-0.12, $P = .0202$) and delayed memory (β = .089, 95% CI 0.032-0.146, $P = .0024$) performance across time (48).

Similarly, 1 study included 487 individuals without cognitive impairment from Spain, of whom 148 had a medical history of T2D. A better score was reported in memory (Cohen's $d = 0.38$, 95% CI −0.02 to 0.79; $P = .115$), executive functions ($d = 0.51$, 95% CI −0.06 to 1.08; $P = .086$), and global cognition ($d = 0.48$, 95% CI −0.01 to 1.04; $P = .124$) in metformin-treated patients at baseline. Nevertheless, these results were not maintained after 3 years of follow-up (47). Consistent with these findings, other research reported a higher performance in verbal memory ($P < .001$), working memory ($P < .01$), and executive function ($P < .01$) in 23 patients with T2D exclusively using metformin treatment. The latter subjects studied were selected from an Australian population-based cohort study, which included 1814 individuals aged 65 69 years (49).

A 16-week placebo-controlled crossover study was conducted on 20 subjects without diabetes with mild cognitive impairment or mild dementia from the United States. This research reported that metformin can pass through the blood–brain barrier after measuring the levels of metformin in cerebrospinal fluid (1 measure at baseline and the other at week 8). Besides, metformin was linked to better executive function (Trail Making Test Part B [TMT-B]: $P = .03$) and trends suggested improvement in learning/memory (Paired Associates Learning: $P = .06$) and attention (Delayed Matching to Sample: $P = .07$) (50). Additionally, 2 other studies showed an improvement in executive function after using metformin. The first was conducted in Canada with 24 surviving pediatric subjects with brain tumor, and the other in the United States with 2925 participants (30, 51).

Lastly, in another small study including 80 subjects with T2D and mild cognitive impairment in the United States (40 metformin-treated and 40 placebo-treated subjects), after controlling for the baseline Disease Assessment Scale-cognitive subscale, the metformin group showed a considerable improvement in the memory test ($P = .02$) (46). In conclusion, the positive effects of metformin have been related to a lower incidence of dementia and other neurodegenerative diseases, as well as improved cognition, primarily in subjects with T2D.

No Effects or Negative Effects of Metformin on Cognition

On the other hand, 1 study conducted in the United States on 2280 participants, of whom 776 were in the metformin intervention arm, reported no significant effects of metformin on cognition (54). Another study of 333 US women who were overweight or obese breast cancer survivors did not demonstrate metformin's effect on cognition (55). A prospective study in 508 patients with T2D did not note a correlation between metformin and a higher score in cognitive tests during an average follow-up of 3.7 years. Moreover, metformin was associated with an enhanced risk of mild cognitive impairment (subhazard ratio 2.75; 95% CI 1.64-4.63, $P < .001$) (52).

Further research included 732 participants from South Korea, of which 93 subjects had T2D with 2.9 years of follow-up. This research outlined that metformin was related with fast decline of Mini-Mental State Examination scores (OR 4.47, 95% CI 1.24-16.05, $P = .022$) and Verbal Immediate Recall scores (OR 7.37, 95% CI 1.19-45.56, $P = .032$) (53).

These mixed effects of metformin on cognition could be due to differences in sample size. Most studies showing positive associations between metformin and cognition have large sample sizes, with many including between 100 000 and 500 000 participants. However, the 2 studies where no results were found between metformin use and cognitive function had small sample sizes ($n = 333$ and $n = 776$) and shorter follow-up periods than those where there were significant results.

The study that reported an association between metformin and rapid mild cognitive impairment had only 200 metformin-treated participants. Additionally, metformin-treated subjects had other associated comorbidities such as hypertension, stroke, and coronary artery bypass grafting. These diseases have a detrimental impact on cognitive function and serve as significant risk factors for dementia (56, 57), making them major confounders in the analysis. Nevertheless, metformin treatment has been shown to decrease serum vitamin B12 concentrations (58). Vitamin B12 depletion could increase the risk of cognitive impairment (59); thus, it could be a parameter to monitor in patients with T2D treated with metformin.

In general, none of the studies investigating the relationship between cognition and metformin considered the results individually in men and women, despite the known sex disparities in neurobiology and cognitive function (60). Other important factors such as nutrition, physical activity, or episodes of hypoglycemia were not considered and could also explain the inconsistencies between the studies. Therefore, further large-scale longitudinal studies are needed to elucidate the potential benefits of metformin on cognition in humans.

Impact of Metformin on Gut Microbiome in Human Studies

Some studies have indicated that the intestine, and not the liver, is a primary target of metformin action. Contrary to oral administration, intravenous metformin did not show glucose-lowering effects (61-63). Metformin reaches 30- to 300-fold higher concentrations in jejunal tissue than in plasma concentrations (64-66). *Caenorhabditis elegans* engages in a beneficial interaction with *E. coli*, a comparable but much simpler relationship than the vast bacterial communities with positive effects that reside in the human gut. Metformin slows the aging process in *C. elegans* only if gut bacteria are present (67). Thus, the effects of metformin on host physiology seem to be possibly regulated by its interaction with the gut content, including the gut microbiota and dietary intake.

Changes Induced by Metformin at the Level of Bacterial Taxonomy

In addition to be the most used oral hypoglycemic agent in T2D, metformin also has potential therapeutic uses (eg, in interstitial lung disease (68), cardiovascular disease (69), in the prevention of hyperlipidemia (70, 71), and aging (72). This wide spectrum of diseases suggests that the effects on gastrointestinal system and the gut microbiota might have a role in the systemic actions of metformin (73, 74). However, human studies on the effects of metformin on fecal microbiota are quite recent, with less than a decade of research. Insulin resistance and T2D have been uncovered to be associated with a dysbiosis of the gut microbiome and a decrease in microbial genetic richness in the context of chronic low-grade inflammation (75).

Bacterial taxonomy, bacterial functions, and metabolomics are affected by metformin (Fig. 2). The most consistent findings across the studies related to changes in bacterial composition were the increased abundance of bacteria belonging to the Enterobacteriaceae family (75-77), specifically the *Escherichia/Shigella* genera, mainly *Escherichia coli* species (20, 22, 75, 78-83), and the increased abundance of *Akkermansia muciniphila* species (22, 83, 84). In addition, another consistent finding was the decrease in the *Intestinibacter bartlettii* species (20, 22, 78, 81, 83). Metformin use was also associated with an increase in *Ruminococcus torques* abundance, although only in 1 study (80). In contrast, metformin use was associated with a decrease in the abundance of genera such as *Romboutsia* (75, 81), *Clostridium* (20, 78, 81, 82), *Roseburia* (20, 80),

Table 2. Human studies focused on metformin, bacterial taxonomy, and functionality

Reference and Clinical Trials.gov identifier	Country	Study design	Findings related to bacterial taxonomy	Bacterial functionality
(78) (NCT02546050)	Denmark	27 healthy men (mean age 26 ± 3.4)	Genera: *Intestinibacter* ↓[a], *Clostridium* ↓, *Terrisporobacter* ↓, immediately after metformin and all the intervention periods. *Senegalimassilia* ↓, and *Lachnospiraceae* ↓, temporarily. *Escherichia/Shigella* ↑[b], immediately after metformin and all the intervention periods. *Bilophila* ↑, *Lachnoclostridium* ↑, *Caproiciproducens* ↑, *Tyzzerella* ↑, and *Prevotella* ↑, at different time periods.	NR[c]
(22)	Spain	18 recently diagnosed T2D subjects with placebo (mean age 54.9 ± 1.9) 22 recently diagnosed T2D subjects with metformin (mean age 52.6 ± 2.0)	In metformin-treated group compared with the placebo-treated group: Genera: *Escherichia* ↑, *Bifidobacterium* ↑, *Intestinibacter* ↓. Species: *Akkermansia muciniphila* ↑, *Bifidobacterium adolescentes* ↑, *Escherichia coli* ↑.	Fecal propionate, butyrate, lactate, succinate ↑, Plasma bile acids ↑, in the metformin group.
(79) (NCT01471275)	China	100 subjects with T2D and metformin (mean age 58.55 ± 9.17). 100 subjects with T2D and herbal formula (mean age 59.00 ± 9.46).	In metformin-treated group compared with the herbal formula-treated group: Alpha-diversity (Simpson's diversity index): ↑. Genera: *Erysipelotrichaceae incertae sedis* ↑, *Escherichia/Shigella* ↑, *Fusobacterium* ↑, *Flavonifractor* ↑, *Lachnospiraceae* ↑, *Lachnospiracea incertae sedis* ↑, and *Clostridium XVIII* and *IV* ↑, *Blautia* ↑, *Anaerostipes* ↑, *Bacteroides* ↓, *Parabacteroides* ↓, *Oscillibacter* ↓, *Alistipes* ↓, *Ruminococcaceae* ↓.	NR
(75)	Latvia	18 healthy subjects treated with metformin (mean age 25.5 ± 7.5)	Alpha-diversity (Shannon index) ↓. Order: Enterobacteriales ↑ Family: Peptostreptococcaceae ↓, Clostridiaceae_1 ↓, Enterobacteriaceae ↑. Genera: *Peptostreptococcaceae*_unclassified ↓, *Clostridiaceae_1*_unclassified ↓, *Asaccharospora* ↓, *Romboutsia* ↓, *Escherichia-Shigella* ↑.	NR
(80)	US	121 subjects (mean age 60 years)	In metformin-treated group: Genus: *Roseburia* ↓. Species: *Escherichia coli* ↑, *Ruminococcus torques* ↑, *I. bartlettii* ↓, *Roseburia intestinalis* ↓, *Roseburia faecis* ↓.	Increased serum acetate, butyrate, and valerate at 6 months, in the metformin-treated group.
(81) NCT03809260	South Korea	20 healthy adult male subjects (between 19 and 33 years old)	Alpha-diversity (Kruskal–Wallis test) ↑, beta diversity ↑. Genera: *Intestinibacter* ↓, *Clostridium* ↓, *Romboutsia* ↓, *Escherichia* ↑.	Urine and plasma: Palmitoleic acid ↓, most carbohydrates ↓, amino acids ↑ post metformin period.
(85)	China	22 subjects with T2D (mean age 55 years)	Alpha-diversity (Shannon index) ↓. Genus: Bacteroides ↓. Species: *Bacteroides finegoldii* ↓, *B. fragilis* ↓.	Serum and stool: Tauroursodeoxycholic acid ↑, conjugated secondary bile acids ↑, glycoursodeoxycholic acid ↑.
(20)	Denmark	277 nondiabetic individuals, 75 T2D subjects, and 31 Type 1 Diabetes subjects from a Danish cohort.	T2D without metformin treatment: Genera: *Roseburia* spp ↓, *Subdoligranulum* sp ↓, *Clostridiales* spp ↓, *Lactobacillus* spp ↑.	↑ butyrate and propionate production.

(*continued*)

Table 2. Continued

Reference and Clinical Trials.gov identifier	Country	Study design	Findings related to bacterial taxonomy	Bacterial functionality
		53 T2D female subjects, and 92 nondiabetic individuals from Swedish. 71 T2D subjects, and 185 nondiabetic individuals from China.	T2D with metformin treatment: Genera: *Escherichia* spp ↑, *Intestinibacter* spp ↓.	
(86)	Latvia	35 healthy nondiabetic individuals (mean age 31.5 ± 10.2). 50 newly diagnosed T2D patients (mean age 58.6 ± 12.5).	Alpha-diversity ↓ in T2D patients. Metformin induced changes: Genus: *Oscillibacter* ↑. Species: *Clostridium bartlettii* ↓, *Barnesiella intestinihominis* ↓, *Parabacteroides distasonis* ↑.	In healthy subjects: folate biosynthesis ↓, thiamine and vitamin B6 biosynthesis ↑.
(84)	Colombia	28 T2D patients, 14 with metformin (mean age 50 ± 10) and 14 without metformin (mean age 44 ± 9) 84 nondiabetic individuals (mean age 47 ± 9)	T2D patients with metformin compared with nondiabetes: Class: Mollicutes ↑(OTU284 y OTU067)). Genera: *Butyrivibrio* ↑(OTU062), *Prevotella* ↑(OTU028), *Megasphaera* ↑(OTU069), *Bulleidia P*-1630-c5 ↑ (OTU077). Species: *Bifidobacterium bifidum* ↑, *Akkermansia muciniphila* ↑. Enrichment of *Prevotella* and *Megasphaera* OTUs was observed.	NR
(76) (NCT02405806)	Sweden	30 T2D patients (mean age 57.5 ± 8.2)	In T2D patients with metformin (n = 23): Family: Enterobacteriaceae ↑.	SCFA concentrations reported no related to metformin.
(87)	China	51 T2D patients with metformin treatment (mean age 58.1 ± 9.4), 36 T2D patients with insulin treatment (mean age 59.75 ± 13.0), 17 T2D patients with α-glucosidase inhibitor treatment (mean age 62.3 ± 10.4), 26 T2D nontreated patients (mean age 56.4 ± 10.6), 50 nondiabetic individuals (mean age 58.5 ± 3.0).	In T2D patients with metformin: Alpha-diversity (Shannon index): ↓. Phylum: Actinobacteria ↑. Class: *Erysipelotrichi* ↓, *Alphaproteobacteria* Genus: *Fusobacterium* ↑, *Spirochaete* ↑, *Turicibacter* ↑, *Ruminococcus* ↓.	Metformin promoted lipids biosynthesis. Also, contributed to the hypotaurine and taurine metabolism.
(77)	Japan	50 T2D patients (mean age 62.5 ± 10.8), 50 nondiabetic individuals (mean age 60.2 ± 12.9).	In T2D patients with metformin: Family: Enterobacteriaceae ↑. Genus: *Staphylococcus* ↑.	NR
(82)	Sweden	53 women with T2D (mean age 70.5 ± 0.1), 49 women with impaired glucose tolerance (mean age 70.5 ± 0.1), 43 women with normal glucose tolerance (mean age 70.3 ± 0.1).	In women with T2D taking metformin treatment. Family: Enterobacteriaceae ↑. Genera: *Escherichia* ↑, *Shigella* ↑, *Klebsiella* ↑,*Salmonella* ↑, *Clostridium* ↓, *Eubacterium* ↓.	NR
(21) (NCT01357876)	United Kingdom	12 T2D subjects (mean age 56 ± 5.4)	On visits with metformin: Genus: *SMB53* ↓, *Adlercreutzia* ↓, *Eubacterium* ↑.	Serum bile acids ↓. Fecal bile acids ↑. Glucagon like peptide 1 (GLP-1) ↑.
(88)	Netherlands	24 T2D patients, 15 metformin users, 9 metformin users.	In T2D patients with metformin: Species: *Escherichia coli* ↑.	SCFA levels ↑ (specially propionate).

Abbreviations: T2D, type 2 diabetes; OTU, operational taxonomic unit.
[a]↓ (decreased).
[b]↑ (increased).
[c]Not reported.

and *Roseburia intestinalis* species (80), among others (Table 2).

Bacterial functionality analysis demonstrated that indirect effects of metformin treatment, such as reduced gut lipid absorption and local inflammation triggered by lipopolysaccharide, could provide a competitive advantage to *Escherichia* spp. This enrichment of *Escherichia* species could trigger a positive feedback loop that further perpetuates the observed taxonomic changes (20). Metformin also leads to a reduction of fecal microbiota diversity, creating the perfect niche for *Escherichia/Shigella* bacteria. Interestingly, some studies found a higher initial presence of *Escherichia/Shigella* spp. in the samples from participants who later manifested adverse effects (75).

Dysbiosis of the gut microbiota, with the predominance of lipopolysaccharide-synthesizing bacteria, such as *Escherichia/Shigella*, could enhance intestinal permeability and bacterial translocation to the systemic circulation and worsening insulin resistance (89). In fact, the increased abundance of *Escherichia/Shigella* was positively correlated with the levels of these proinflammatory cytokines (IL-6, CXCL2, NLRP3, and IL-1β) (90-93). Importantly, the presence of bacterial endotoxins has been demonstrated in typical senile plaque lesions in Alzheimer disease brains (94). Thus, it seems contradictory that the enrichment of *Escherichia/Shigella* genera could have beneficial effects. A detailed mechanistic investigation suggests agmatine as an *E. coli*-produced metabolite necessary for the effects of metformin on host lipid metabolism and lifespan in both *C. elegans* and *Drosophila melanogaster* (73). The study demonstrated a causal link between metformin supplementation and agmatine production by *E. coli* to increase host lifespan, with the phosphotransferase system–*Crp* bacterial axis as a central regulator of the effects of metformin on the host. Metformin treatment was also associated with a predicted increase in agmatine production capacity by *E. coli* and other Enterobacteriales in subjects with T2D (73). However, it is not possible to determine if the rise in agmatine in metformin-treated patients with T2D is linked with an increase in longevity when compared with patients with T2D not taking the medication. Hence, the interaction of metformin with the gut microbiota (with dietary nutrients as a critical factor) could contribute to the beneficial effects and negative side effects observed with metformin use.

Some authors demonstrated that metformin enhances the abundance of *E. coli* while impairing *Intestinibacter bartlettii* growth (80). Functional analysis of *I. bartlettii* reveals that it can degrade fucose, implying an indirect role in mucus degradation (80). *I. bartlettii* has also been linked to changes in propionate metabolism (83), but the effects of the *Intestinibacter* genus on human health are uncertain (20).

In contrast, *Akkermansia muciniphila* is a bacterium specialized in mucin degradation. It also strengthens the integrity of the intestinal epithelium, regulates intestinal barrier function, and is important in glucose homeostasis (95). Some human studies have reported an increase in the abundance in *Akkermansia muciniphila* after metformin treatment (22, 83, 84). Additionally, metformin increases the growth of this bacterium in pure cultures. *A. muciniphila* was negatively associated with low-grade inflammation, T2D, and insulin resistance in mice (96). Likewise, in mice treated with metformin, an increase in the proportion of *A. muciniphila* and a positive correlation with the number of mucin-producing goblet cells have been demonstrated. Therefore, an increase in the mucus layer by goblet cells could provide a barrier to lipopolysaccharide (97-99). Besides, the administration of metformin exhibited a notable effect on the relative abundance of *A. muciniphila* in aged mice. This, in turn, led to a significant reduction in the levels of the systemic inflammatory biomarker IL-6 (100). Excessive production of peripheral IL-6 has been observed to contribute to elevated IL-6 levels within the brain. Systemic production of IL-6 can potentially cross the blood–brain barrier and, upon reaching the brain, may have a detrimental impact on neurogenesis (101, 102). Overproduction of IL-6 impairs neurotransmission in brain regions that modulate cognition, such as the hippocampus and prefrontal cortex (103, 104).

Despite these results in animal models, there is no evidence that *A. muciniphila* is associated with improved glycemic control in humans, furthermore, little or no evidence suggests a causal role of *A. muciniphila* on the control of obesity/diabetes in humans. One study in humans did not find associations between changes in *A. muciniphila* abundance and glycated hemoglobin levels (22). Other studies in humans did not report increases in the *A. muciniphila* abundances related to metformin use (75, 78, 79). These differences may result from individual factors such as age (105, 106), immune response (107, 108), and fiber intake (109), or polyphenol availability (110, 111). Although the direct effect of metformin on *A. muciniphila* has not been fully characterized in humans, the increased abundance of *A. muciniphila* due to metformin treatment could restore the increased intestinal permeability induced by T2D and high-fat diets.

On the other hand, the increase in *Ruminococcus torques* abundance induced using metformin has been associated with an increase in SCFA production (80). In contrast, metformin use was associated with decreases in the abundance of the *Roseburia* genus (20, 80) and *Roseburia intestinalis* species (80). The genus *Roseburia* comprises 5 clearly characterized species, including *Roseburia intestinalis*, all of which produce SCFA. Specifically, *Roseburia intestinalis* is a major producer of butyrate. The abundance of this genus has decreased in subjects with Parkinson disease. Contradictory results have been found with major depressive disorder, where both increases and decreases in abundance have been seen.

The inconsistencies between studies could be determined because metformin dose, time of drug use, study duration, presence, or absence of T2D, time of diagnosed T2D, racial differences, and sample size. Notably, some of these studies employed 16S ribosomal (rRNA) sequencing to analyze the composition of the gut microbiota. 16S rRNA introduces systematic biases, copy number variations, and variability in polymerase chain reaction amplification. Hence, the shotgun metagenomics approach is more accurate in functional characterization of the microbial community, according to strains, species, and their biological role (112).

Metformin-Induced Changes in Metagenomics Functions and Metabolomics

Over the past decade, numerous studies have shown that the effects of bacterial composition and its function are reflected in the circulating metabolome. Regarding functional analysis, the major findings are related to acetate, lipopolysaccharide, peptidoglycan synthesis pathways, glucose metabolism pathways, and bile acids (20, 22, 80, 81, 85). In addition, the main findings concerning metabolomics are related to variable alterations in the concentrations of short-chain fatty acids, hippuric acid, and amino acids (20-22, 76, 80, 85, 87). The

2 most investigated results in the literature are related to increased concentrations of SCFA and bile acids.

Findings related to short-chain fatty acids

One of the best characterized functions of the gut microbiota is the production of SCFA through the fermentation of nondigestible carbohydrates derived from the diet. The hypothesis that metformin partly exerts its beneficial effect on cognition is related to SCFA biological functions. SCFA inhibit neuroinflammation and regulate enteric nervous system, and are involved in brain function, neuroplasticity, and behavior. Furthermore, SCFA regulate neurotransmitter synthesis and the expression of receptors, such as dopamine and γ-aminobutyric acid receptors (113, 114). Therefore, disequilibrium in SCFA production could play a determinant role in the development of cognitive impairment and neurodegenerative diseases.

The SCFA (acetate, propionate, and butyrate) have a crucial role in controlling metabolism and supply of energy, in addition to preserving the homeostasis of the gut environment. Acetate is categorized as a dominantly obesogenic SCFA because it acts as a substrate for the synthesis of cholesterol and fatty acids in the liver and other tissues. Propionate has been described to protect from diet-induced obesity by upregulating the expression of the gene that encodes leptin synthesis. Propionate has gluconeogenic effects in the liver, whereas acetate and butyrate are lipogenic. Butyrate is involved in the regulation of colonic mucosal homeostasis, cell proliferation, and differentiation, enhancing insulin sensitivity. Butyrate has been also shown to increase energy expenditure and protect colonocyte membrane function by limiting oxidative stress (4, 115, 116).

A multicountry metagenome dataset in subjects from Denmark, Swedish, and China (199 subjects with T2D, 31 subjects with type 1 diabetes, and 554 subjects without diabetes) showed that patients with T2D without metformin treatment showed a decrease in the abundance of genera containing butyrate-producing species such as *Roseburia* and *Subdoligranulum*. In contrast, in metformin-treated subjects, a notable increase in the *Subdoligranulum* genus was observed. The final analysis reported a rise in butyrate and propionate production in those treated with metformin (20).

Likewise, SCFA production in 24 Dutch patients with T2D (9 without metformin and 15 using metformin) was compared. SCFA levels were shown to be higher in patients with T2D who were not taking metformin, particularly butyrate (88). In another study, a group of 18 subjects with newly diagnosed T2D treated with placebo was compared with another of 22 subjects with newly diagnosed T2D treated with metformin recruited in Spain. In the metformin-treated group, elevated fecal concentrations of butyrate and propionate were observed in men. However, no significant results were obtained when men and women were analyzed together (22).

Lastly, 121 overweight/obese subjects were studied in the United States. Those individuals taking metformin displayed higher acetate and butyrate levels at 6 months, but these results were not sustained at 12 months. In the same way, metformin raised SCFA-producing *Ruminococcus torques* and SCFA production pathways (80).

Thus, metformin appears to exert beneficial effects on neuroinflammation and glucose homeostasis, either by a direct effect on SCFA-producing pathways or by increasing the SCFA-producing gut microbiota. However, the exact mechanism whereby metformin modulates both gut microbiota and metabolic homeostasis remains unclear.

Metformin and microbiota functions related to bile acid metabolism

Bile acid biosynthesis comprises 2 mechanisms and anatomical sites. The first is the de novo synthesis of primary bile acids from cholesterol in the liver, and the second is the formation of secondary bile acids because of bacterial enzyme modification of primary bile acids in the gut (117). Primary bile acids can be deconjugated, oxidized, and epimerized, or dehydroxylated, by the gut microbiota to create secondary bile acids. Because deconjugated primary bile acids can operate as signaling molecules that alter the overall bile acid pool, the microbiota could have evolved the deconjugation mechanism to further modulate bile synthesis. Increased concentrations of antimicrobial bile acids, cholic acid, and chenodeoxycholic acid also result from deconjugation, which may cause modifications in microbiome composition (117, 118). All major bacterial phyla such as contain enzymes (bile salt hydrolases) capable of performing the deconjugation process, implying that the genes encoding these enzymes (*bsh* genes) are horizontally transferable (117-119). Bile salt hydrolases can deconjugate glycine- and taurine-bound primary bile acids. The gut microbiota can also employ the released residues of glycine and taurine as nutrient resources; thus, deconjugation is a gut microbiome critical function (117).

In contrast, epimerization requires the actions of α-and β-bile acid hydroxysteroid dehydrogenases (HSDHs) (118). 3α- and 3β-HSDHs have been found in the gut microbiota of various Firmicutes phylum, although intraspecies 3-hydroxy epimerization has only been identified in *Peptostreptococcus productus*, *Clostridium. perfringens*, and *Eggerthella lenta* (117, 120). 7α-HSDHs are identified among members of the genera *Clostridium*, *Eubacterium*, *Bacteroides*, and *Escherichia*, while 7 β-HSDHs are exclusively found in Firmicutes. Species belonging to *Clostridium*, *Eubacterium*, and *Ruminococcus* genera are the only ones capable of intraspecies 7-epimerization (118, 121). Lastly, due to the inaccessibility of the hydroxyl group, dehydroxylation can only occur after deconjugation. Species with 7α-dehydroxylation activity belong to the Firmicutes phylum (*Clostridium* (*C. scindens* and *C. hylemonae*), and *Eubacterium* genera) (118).

Bile acids play a key role in glucose, energy, and lipid homeostasis. The modulating effects of bile acids on various metabolic pathways occur primarily through intracellular nuclear receptors binding, among them FXR. FXR is a ligand-activated nuclear receptor that modulates hepatic bile acid metabolism and is implicated in several metabolic diseases (122, 123).

Glycoursodeoxycholic acid (GUDCA) is a bile acid obtained by the conjugation of ursodeoxycholic acid (secondary bile acid) and glycine. GUDCA has been linked to a neuroprotective function due to its antiapoptosis, anti-inflammatory, and antioxidant effects. Its role in metabolic disorders is starting to be examined because the interaction of GUDCA and intestinal FXR could reduce body weight gain and improve glucose intolerance as well as insulin resistance (122, 123). In this regard, metformin treatment raised bile acid GUDCA levels in the gut in 12 T2D patients. Therefore, metformin

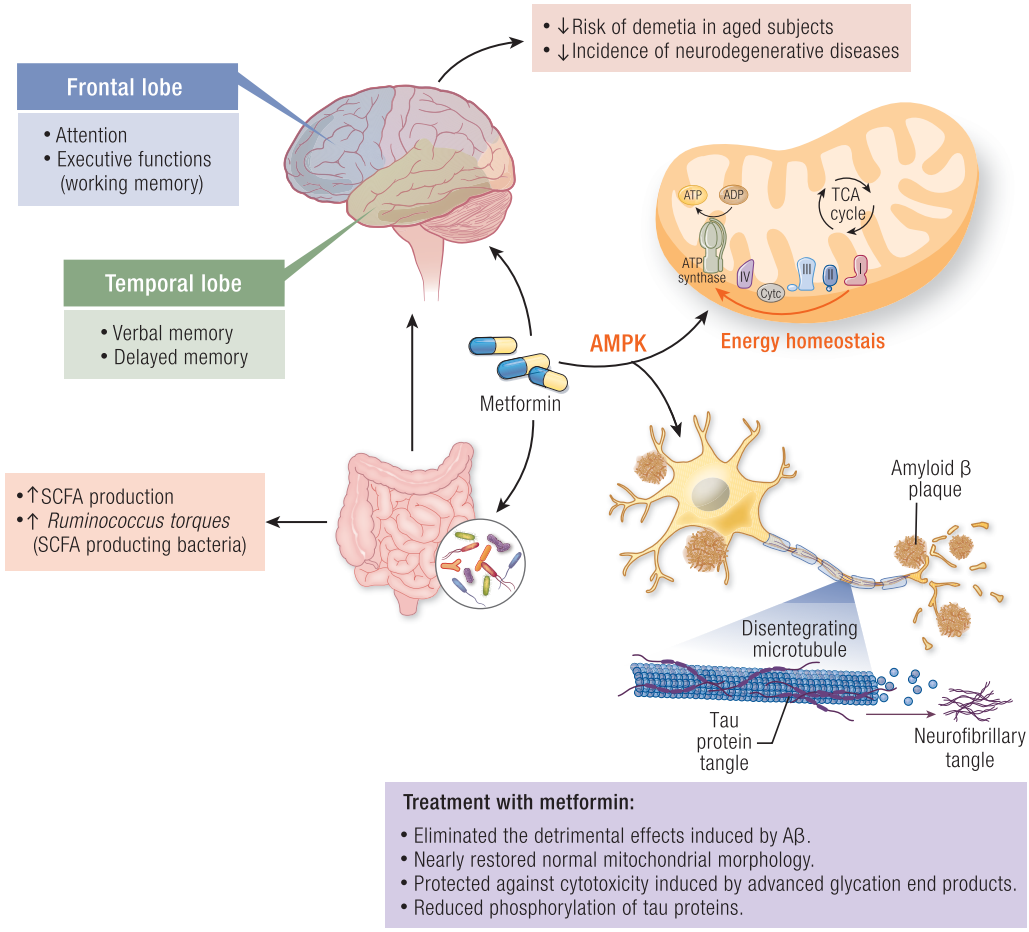

Figure 1. Impact of metformin on cognition. Each chart shows the cognitive domain where metformin has been reported to have effects and the brain region involved in each cognitive function. Clinical and preclinical studies show that metformin has a protective effect against dementia and decreases the incidence of neurodegenerative diseases such as Alzheimer disease. Although the mechanisms by which metformin has neuroprotective effects are not fully elucidated, metformin primarily exerts its effects on energy homeostasis within neurons by targeting AMPK. Furthermore, the effects of metformin on cognition could be facilitated through the gut microbiota. Aβ, amyloid-beta; AMPK, adenosine monophosphate-activated protein kinase.

might inhibit bile acid reabsorption, leading to a longer exposure time of the intestine to bile acids and an increase in the concentration of bile acids in the feces. Prolonged exposure to bile acids could result in the binding of bile acids to intestinal FXR (21).

Consistently, a study conducted in China with 22 patients with T2D, metformin therapy raised GUDCA levels by decreasing the abundance of *B. fragilis* and the activity of its bile salt hydrolase. Metformin restrained the growth of *B. fragilis* by affecting folate and methionine metabolism. This study demonstrated that enhancements of glucose metabolism in metformin-treated mice were transferred by *B. fragilis* colonization (85).

Consequently, 1 survey reported a raise in the plasma bile acid (primary, secondary, and unconjugated) concentration after 4 months of metformin treatment in 22 patients with T2D. Also, an increase in the abundance of *bsh* genes was described after 2 months of metformin treatment. Hence, an increase in *bsh* genes could lead to an increment in the concentration of unconjugated bile acids. Furthermore, this study found a negative correlation between glycated hemoglobin and unconjugated bile acids concentrations (22). Therefore, the effects of metformin on glucose metabolism could be mediated by an AMPK-independent pathway through the intestinal FXR, *B. fragilis*, GUDCA, and bile acids.

Clinical Implications of the Metformin-Induced Changes in the Gut–Brain Axis

Neurodegenerative diseases and cognitive impairment pose a therapeutic and diagnostic challenge. The risk of developing these pathologies increases the longer a person lives with T2D, especially if there has not been adequate metabolic control (124). To date, metformin has been proposed to show neuroprotective, anti-inflammatory, and antioxidant effects and its use has been associated with a lower risk of dementia in subjects with T2D (27, 28, 42, 44). Likewise, improved memory has been demonstrated in patients with mild cognitive impairment and T2D who regularly use metformin (46, 48). Also, a lower incidence of neurodegenerative diseases has been described in older adults with T2D and metformin treatment (44). Specifically, metformin has been associated with better cognition, hence, better executive function (30, 42, 47, 49), memory (47, 49) and semantic memory (49) in subjects with T2D. In subjects without diabetes with mild cognitive impairment, metformin use has also been associated with improved attention and executive function (50).

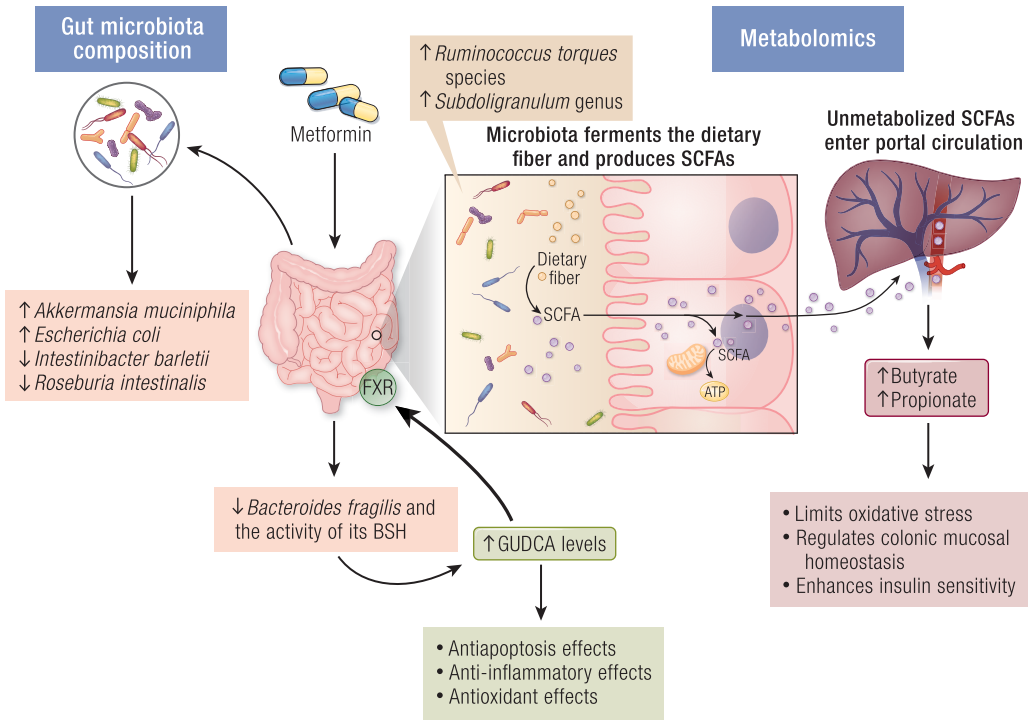

Figure 2. Impact of metformin on gut microbiota. The figure shows the main effects of metformin on the gut microbiota that have been reported in clinical studies. GUDCA, glycoursodeoxycholic acid; BSH, bile salt hydrolase.

Even better cognitive performance has been observed after the use of metformin in pediatric brain tumor survivors (51). Despite these encouraging results, an association between metformin and cognitive impairment has also been reported (53). Part of the metformin's beneficial effects could be modulated by the gut microbiota composition and functionality, with an increase in the production of SCFA and bile acids.

Despite the encouraging results, most studies investigating the metformin–gut-microbiota–brain axis in humans are mainly associative in nature, except for 1 study that employed transplantation of human gut microbiota into germ-free mice and a gut simulator. In this study, the transfer of metformin-altered fecal microbiota to germ-free mice demonstrated that gut microbiota–metformin interaction is required to improve glucose metabolism. Moreover, when feces were cultured in vitro with metformin in a simulated gut environment, transcriptome analyses revealed direct effects of metformin on the microbiota. These effects included the regulation of gene expression in gut bacteria encoding metalloproteins (22).

Human research is not progressing as fast as animal research. Nevertheless, human research on the gut microbiota-gut–brain–metformin axis has reported interesting and consistent associations. Clinical research has demonstrated important differences in cognition as well as gut microbiota composition and metabolomics when comparing individuals with and without metformin. However, many of the studies are case–control comparisons that do not consider critical confounding factors such as diet.

While animal studies have been instrumental in generating hypotheses and establishing causal relationships, there is still much ground to cover to fully translate these findings to human research. To understand the level of contribution of metformin on gut microbiota and cognition, research on genetic information and risk alleles associated with the diseases of interest will be necessary. Studies with large human cohorts and follow-up over time will be necessary. This will permit the definition of the directionality of cause and effect to generate hypotheses that can be tested in humans and experimental systems. Across cohorts, replication will also be critical. Longitudinal correlation of blood and urinary biomarkers with microbial products involved in cognition and inflammation will be essential to determine cellular and molecular mechanisms. Lastly, intervention studies aimed at directly changing the gut microbiota will also be worth exploring. Understanding the complex metformin–gut–brain axis interactions could allow us to individualize and target the beneficial actions of metformin as well as diminish its adverse effects.

Conclusions

Metformin is a well-tolerated drug that has been used for T2D treatment since the 1950s. It is widely accepted that the pleiotropic effects of the drug are due to its action on the mitochondria causing a mild and specific inhibition of the respiratory-chain complex 1. However, the exact mechanism of action of this drug remains elusive. Metformin's primary target of action has been identified as the gut, and intriguingly, its ability to slow down the aging process in *C. elegans* is dependent on the presence of gut microbiota. This review highlighted the existing understanding from human studies regarding the effects of metformin on the gut microbiome, the associated alterations in cognitive function, and the potential bidirectional implications of these interactions.

T2D has been related to impaired memory, executive functions, and an increased risk of dementia. Metformin could have benefits on cognition through several pathways, decreasing glycation end products, inflammation, and preventing the

development of the metabolic syndrome. Metformin treatment has been reported to have mixed effects on cognition. Despite there being studies where metformin shows a positive impact on memory, attention, and executive functions, some studies showed no significant effects of metformin on cognition or even an enhanced risk of mild cognitive impairment. However, the precise mechanisms underlying the neuroprotective effects of metformin or its potential to enhance cognitive performance across various cognitive domains remain elusive.

Several studies have demonstrated that metformin exerts changes in the composition of the gut microbiota taxonomy, function and in the metabolome. Metformin seems to fundamentally affect the bacterial composition of patients with T2D, resembling the composition of the gut microbiota of patients without T2D. In addition, it has effects on several metabolic synthesis pathways, the concentration of bile acids, and SCFA. In parallel to metformin, there are other factors that influence the gut microbiota such as medications, sex, diet, body mass index, and other diseases. It is vital to control for confounding factors to ensure that the observed results in studies evaluating metformin effects are in fact attributable to metformin.

Despite its cost, the study of the fecal microbiota by means of shotgun metagenomics is increasingly important. To elucidate the effects of metformin on gut microbiota, it is essential to understand the functional characterization of the microbial community (strains, species) as well as to infer its biological role and metabolic phenotype. Transplantation of fecal microbiota from humans to mice could also be necessary to validate metformin's mechanism of action and findings. To our knowledge, no studies have investigated the interrelationship among metformin, gut microbiota, and cognition. Thus, it will be of interest to explore whether the effects of metformin on cognition could be mediated or regulated by the gut microbiota.

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Author Contributions

J.M.F.-R. conceptualized and designed research; M.R.D. drafted manuscript and prepared figures; J.M.F.-R. edited and revised manuscript; all authors approved the final version.

Disclosures

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