Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Probiotics

Evidence Summary

Probiotic strains can influence host physiology, particularly immune responses and metabolism via bioactive metabolites and altering the microbiome, but have minimal impact in the already healthy.

Neuroprotective Benefit: Through production of neuroactive metabolites some probiotic strains can affect brain activity, especially for emotional processing, and may alleviate the negative effects of stress.

Aging and related health concerns: Probiotic strains have immunomodulatory properties, and can impact metabolic parameters, but the effects are dependent on interactions with the host microbiome and physiology.

Safety: Probiotics are generally safe, but due to their activity in gut, may produce temporary discomfort in the gastrointestinal system. Probiotics-related infections are rare, but can occur in high-risk immunocompromised individuals.

Availability: OTC	**Dose**: Not established
Half-life: N/A	**BBB**: Probiotic strains reside in the gut, but some of their metabolites are BBB penetrant
Clinical trials: Hundreds of RCTs generally on the order of 20-100 participants/study have been conducted with various probiotic strains.	**Observational studies**: 10-year Finnish and 6-year Swedish studies found no evidence for increases in probiotic strain-related bacteremia with probiotic use in the general population.

What is it?

Probiotics are defined by the World Health Organization (WHO) as "live organisms which when administered in adequate amounts confer health benefits to the host" [1]. These microorganisms, usually strains of bacteria, but also fungi, such as yeast, are ingested in the form of supplements or as part of fermented foods. While this definition indicates the use of live organisms, there is some evidence that dead strains may also confer benefits under some conditions [2]. Since probiotics are regulated as supplements and food products, there are no clear standards regarding the strains that can be used, the amount contained in a product, or the activity level/bioactive properties of the strains [3]. This has influenced the clinical evaluation of probiotics in a manner which hampers the identification of the most clinically efficacious probiotics, as the composition and formulation of the probiotics varies across studies.

Probiotics are primarily used to restore disruptions to the homeostasis of the gut microbiota [4]. Changes to the gut microbiota impact the metabolism of the host, and dysbiosis of the gut microbiota is a common feature of aging and chronic diseases. The microorganisms in the gut produce a variety of bioactive metabolites which can influence host physiology, especially with respect to the immune system. The major goal of probiotic treatment is the rejuvenation of the gut microbiome toward a healthy state. The ability of a given probiotic strain or set of strains to exert a therapeutic effect is highly dependent on the baseline microbiota of the host, as well as other host-related physiological factors [5]. Consequently, different probiotic preparations will be more or less efficacious in different people, and there is no universally beneficial probiotic. Probiotics have been tested in numerous conditions including, but not limited to, gastrointestinal disorders, metabolic disorders, immune dysregulation, cardiovascular diseases, and cognitive impairment. Improvements in clinical trial design, such as the

inclusion of biomarkers to assess the relationship between a given probiotic's metabolic or immunomodulatory effects and efficacy on clinical metrics, may facilitate the identification of probiotic products with therapeutic utility.

Neuroprotective Benefit: Through production of neuroactive metabolites some probiotic strains can affect brain activity, especially for emotional processing, and may alleviate the negative effects of stress.

Types of evidence:

- 2 meta-analyses of RCTs of probiotics in healthy volunteers
- 1 meta-analysis of RCTs of probiotics in psychiatric disorders
- 1 meta-analysis of RCTs of probiotics in neurological diseases
- 3 meta-analyses of RCTs of probiotics in dementia
- 2 RCTs of probiotics for cognitive function in fibromyalgia
- 3 RCTs of probiotics for cognitive function in healthy adults
- 2 RCTs of probiotics in Parkinson's disease
- 7 RCTs of probiotics in AD/MCI

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

Probiotic use has generally not been associated with significantly improved cognitive function in healthy individuals in clinical trials, however, this is generally attributed to the ceiling effect for the tests used to measure cognitive function in these studies [6]. Performance on emotion-related tasks, is the one area where probiotics appear to benefit cognitive function in stressed, but healthy populations. The protective effects are likely due to the ability of some bacterial strains to produce metabolites which can influence neurotransmitters in regions of the brain associated with emotion processing, as well as metabolites that impact immune function. A variety of studies have found alterations to immune function and/or the inflammatory profile in individuals with mood disorders [7].

In healthy volunteers (n=45) the use of the probiotic Ecologic®825 for four weeks led to changes in fMRI brain activation patterns in response to emotional memory and emotional decision-making tasks, and reduced vulnerability to depression [8]. There were significant changes in brain activity in the anterior cingulum, and these changes were associated with Positive and Negative Affect Schedule (PANAS) positive scores ($r = -0.534$, $P = 0.04$). An RCT testing the probiotic strain Bifidobacterium longum 1714™ (1×10^9 cfu/day) for four weeks in stressed healthy volunteers (n=40) showed evidence for changes in resting-state neural oscillations with probiotic use [9]. There was an increase in the theta

band (6 Hz) power in the bilateral inferior, middle, and superior frontal cortices, as well as in the bilateral anterior and middle cingulate cortex. In RCTs (n=31, 32, respectively) testing a multispecies probiotic for fibromyalgia (ERGYPHILUS Plus), probiotic use was associated with an improvement in attention based on performance on a Go/No-Go Task, and a reduced number of impulse choices [10; 11]. A meta-analysis of 14 studies on the effects of probiotics in sleep found that probiotic use was associated with an improvement in sleep quality based on a reduction in the Pittsburgh Sleep Quality Index (PSQI) score (Mean Difference [MD] −0.78-points, 95% Confidence Interval [CI] 0.395 to 1.166; P < 0.001), but the effects were not significant for other sleep-related parameters [12].

While probiotics may help alleviate some of the cognitive effects of stress, it is less clear whether they can have a meaningful impact on alleviating the mood disorders themselves. A meta-analysis of ten RCTs (n=685 participants) found that probiotic use was associated with a reduction in depression scores (Standardized mean difference [SMD] −0.48, 95% CI −0.71 to −0.26), but had no significant effect on anxiety [13]. The disparate results across studies may stem from the use of different assessments. A meta-analysis of 12 RCTs (n=656 participants) found that probiotic use significantly reduced scores on the Hamilton Depression Rating Scale (Weighted mean difference [WMD] -9.60, 95 % CI -10.08 to -9.11), but had no significant effect on the Beck Depression Index [14]. There is also evidence to suggest that some of the benefits may stem from the immunomodulatory properties of probiotics, as there were changes in some inflammatory markers, such as C-reactive protein (CRP), in this population [14]. While there are variations across studies, the reduction in pro-inflammatory markers has also been seen in stressed healthy populations [15].

Human research to suggest benefits to patients with dementia:

Probiotic use is associated with improved cognitive function in patients with cognitive impairment in clinical trials. In a meta-analysis including seven clinical trials (n=320 participants), probiotic use was associated with enhanced cognitive function in individuals with cognitive impairment (SMD 0.25, 95% CI 0.05 to 0.45, *P* = 0.01) [16]. Though the effects in human studies were modest compared to the cognitive enhancement seen in animal models of dementia (SMD 1.02, 95% CI 0.52 to 1.51, *P* < 0.001). A separate meta-analysis including five RCTs (n=297 participants), three of which were also included in the aforementioned analysis, similarly found that probiotic use was associated with cognitive improvement (SMD 0.37, 95% CI 0.14 to 0.61; P = 0.002) [17]. However, the efficacy was not uniform across trials, as it was influenced by the stage of disease and the duration of intervention. When stratified by length, studies that were shorter than 12 weeks were not associated with cognitive benefits (SMD 0.18, 95% CI −0.08 to 0.44, P = 0.17). A meta-analysis including only the three RCTs (n=161 participants) common to the prior analyses failed to find a significant association between probiotic use and cognition (SMD 0.56;

95%CI −0.06 to 1.18, *P* = 0.07), though the certainty of evidence was very low [18]. The lack of efficacy was driven by a single trial (n=48) conducted in Iran, in which 83.5% of randomized participants had severe Alzheimer's disease (AD), based on Test Your Memory scores [19]. In this study, probiotics did not have significant effects on either cognitive or biochemical measures, while other studies suggest that the effects on cognition are at least partially associated with the changes on metabolic and immune-related parameters.

In an RCT in Iran, AD patients (n=60) treated with probiotic milk (200 ml/day) containing *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*, and *Lactobacillus fermentum* (2×10^9 colony forming units [CFU/g] for each) showed a significant improvement in Mini-Mental State Exam (MMSE) score (+27.90% ± 8.07) relative to the placebo group (−5.03% ± 3.00) after 12 weeks [20]. In people with mild cognitive impairment (MCI) (n=92), a probiotic, DW2009 (800 mg/day), consisting of *Lactobacillus plantarum* C29-fermented soybean (1.25×10^{10} CFU/g) improved the change in the composite score of cognitive functions related to memory and attention, as measured by computerized neurocognitive function tests after 12 weeks [21]. The effect was driven by improvement on the attention domain. Treatment with DW2009 led to an increase in serum levels of brain derived neurotrophic factor (BDNF) relative to the placebo group (412.7 pg/mL ±7212.4 vs −1034.3 pg/mL ± 5644.5), and the change in BDNF level was positively correlated with the effect on cognition. This RCT was conducted in South Korea. In combination with selenium supplementation, a probiotic containing *Lactobacillus acidophilus, Bifidobacterium bifidum*, and *Bifidobacterium longum* (2×10^9 CFU/day) improved MMSE score (+1.5 ± 1.3 vs. +0.5 ± 1.2 and −0.2 ± 1.1, P < 0.001) after 12 weeks in AD patients (n=79) in an RCT conducted in Iran [22]. In a small uncontrolled clinical study in Brazil, AD patients (n=13) treated with probiotic-fermented milk (2 mL/kg/day) for 90 days showed a 28% improvement on the MMSE relative to baseline, as well as improvements in verbal fluency, memory, constructive abilities, and attention [23]. A probiotic containing the strain *Bifidobacterium breve* A1 improved MMSE scores in Japanese patients with MCI (n=19) after 24 weeks in an open-label study [24]. The cognitive enhancing effects of this strain (*Bifidobacterium breve* A1 MCC1274) were confirmed in this population in an RCT (n=79) such that there was a significant improvement on the RBANS relative to placebo after 16 weeks (mean between-group difference 11.3, 95% CI 6.7 to 15.8, P < 0.0001), with significant improvements on the domain scores for immediate memory, delayed memory, and visuospatial [25].

Although the overall evidence suggests that probiotics may be beneficial in the context of MCI or early dementia, due to the considerable variability in probiotic strains or formulation, country of origin, and cognitive assessments used across trials, a consensus on the optimal probiotic for cognitive health

cannot be established. It is likely that different people may preferentially benefit from different probiotic interventions, depending on their physiology and microbiome.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

The mechanisms mediating the effects of probiotics on cognition and mood are not fully understood, however, there are several prevailing hypotheses, based primarily on animal studies.

Neuromodulation: The effects are likely mediated via the gut-brain axis whereby the gut microbiota modulate brain function through the production of bioactive metabolites [6]. For example, microbiome-produced short chain fatty acids (SCFAs) can influence neurotransmission, in part by affecting the synthesis of neurotransmitters, such as norepinephrine, serotonin and dopamine [11]. Notably, these neurotransmitters influence activity in brain areas associated with emotional processing, which may account for some of the effects on mood and attention [8]. Since different bacterial species produce different metabolites, not all strains are equally capable of neuromodulation. Additionally, the colonization of the gut with a probiotic bacterial strain has the potential to alter the levels and/or metabolic activity of other strains within the gut microbiota, some of which may produce their own neuromodulatory metabolites. As a result, the effects depend on the particular probiotic strain used as well as the overall environment/microbiome of the individual's gut [26]. As such, heterogeneity in the response to probiotics is expected depending on both the composition of the probiotic and factors intrinsic to the individual. Unlike other conditions where multi-strain probiotics appear to be more effective, in studies assessing cognition, single-strain studies appear to have a stronger effect [16]. This may be due to the differential neuromodulatory capacity of different strains, such that specific effects may be easier to detect with a single strain, whereas there may be more variability in the response in the context of multiple strains.

Immunomodulation: The gut microbiome is a critical regulator of systemic inflammation, as many of its bioactive metabolites influence immune cell function, which can direct them toward either a pro- or anti-inflammatory state [5]. Reductions to the inflammatory profile and oxidative stress are thought to underlie the majority of the neuroprotective effects of probiotics [20], though there is limited evidence from clinical studies. A meta-analysis of five RCTs found that probiotic use in patients with MCI or AD was associated with a reduction in the systemic inflammatory marker high-sensitivity C-reactive protein (hs-CRP) (SMD −0.57, 95% CI, −0.95 to −0.20, P = 0.003; based on n = 2 studies, n = 57 subjects) [17]. Probiotic use was also associated with a reduction in the oxidative stress marker malondialdehyde (MDA) (SMD −0.60, 95% CI −0.91 to −0.28; P = 0.000; based on n = 3 studies; n = 82 subjects). Inflammatory and oxidative stress markers were also reduced in patients with Parkinson's disease

treated with probiotics for 12 weeks. In one study (n=50), the expression of IL-1, IL-8, and TNF-α were decreased in peripheral blood monocular cells (PBMCs) [27]. In a separate study (n=60), probiotic use reduced hs-CRP (−1.6 ± 2.5 vs. +0.1 ± 0.3 mg/L, P < 0.001) and MDA (−0.2 ± 0.3 vs. +0.1 ± 0.3 µmol/L, P = 0.006), and increased glutathione (GSH) levels (+40.1 ± 81.5 vs. −12.1 ± 41.7 µmol/L, P = 0.03), relative to placebo [28]. Both studies took place in Iran and used a multi-strain probiotic containing *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus reuteri*, and *Lactobacillus fermentum* [27; 28].

Metabolism: Probiotics can influence metabolic parameters. Since metabolic dysfunction is a risk factor for dementia, the modulation of metabolic parameters toward a more healthful state may also reduce cognitive dysfunction. A meta-analysis of three RCTs found that patients with dementia, probiotics reduced plasma triglycerides (MD −19.22 mg/dL; 95%CI −29.79 to −8.64 mg/dL, P = 0.0004), very-low-density lipoprotein cholesterol (VLDL-c) (MD −3.37 mg/dL, 95%CI −5.94 to −0.80 mg/dL, P = 0.01), and insulin resistance (MD −0.32, 95%CI −0.51 to −0.13, P = 0.001) [18]. A meta-analysis of nine RCTs including patients with neurological disorders also found that probiotic use was associated with a reduction in insulin resistance (HOMA-IR) (WMD -0.71, 95 % CI -0.89 to -0.52), triglycerides (WMD -18.38, 95 % CI -25.50 to -11.26) and VLDL-c (WMD -3.16; 95 % CI -4.53, -1.79), and an increase in high density lipoprotein cholesterol (HDL-c) (WMD 1.52, 95 % CI 0.29 to 2.75) [29]. In a recent RCT not included in these meta-analyses, the change in glycated hemoglobin (HbA1c), a measure of glycemic control, was associated with cognitive improvement, based on the RBANS measure of cognitive function (ρ=–0.4218, p=0.006) with probiotic intervention [30]. These studies suggest that the enhancement of cognitive function following probiotic use may be related to an improvement in energy homeostasis leading to better glucose utilization in the brain.

APOE4 interactions: Preclinical studies in rodent models suggest that the presence of ApoE4 influences the microbiome [31], however, the clinical trials conducted thus far have not been stratified by ApoE genotype, so it is unclear whether the presence of ApoE4 influences the efficacy of different probiotic strains.

Aging and related health concerns: Probiotic strains have immunomodulatory properties, and can impact metabolic parameters, but the effects are dependent on interactions with the host microbiome and physiology.

Types of evidence:

- 11 meta-analyses of RCTs on probiotics for metabolic diseases
- 4 meta-analyses of RCTs of probiotics for cardiovascular diseases
- 4 meta-analyses of RCTs of probiotics for kidney diseases

- 2 meta-analyses of RCTs of probiotics for osteoporosis
- 12 meta-analyses of RCTs of probiotics for infections and immune regulation

Metabolic disease: PROBIOTICS MAY REDUCE BMI IN THE OVERWEIGHT

Probiotic use is associated with positive trends on anthropomorphic measures in the context of metabolic-related diseases. A meta-analysis of 61 RCTs (n=5422 participants) testing probiotics or synbiotics (a mixture of probiotics and prebiotics) in otherwise healthy people with cardiometabolic diseases found benefits in the reduction of body mass index (BMI) (SMD −0.156, 95%CI −0.27 to −0.04, P = 0.006; 16 studies, n=1256), and waist circumference (SMD −0.147, 95%CI −0.30 to 0.03, P = 0.05; 8 studies, n=690) [32]. This population also showed reductions in total cholesterol and blood pressure. No clear associations could be made between clinical effects and discrete changes in microbiota composition or immune parameters. Similarly, a meta-analysis of 20 RCTs (n=1411 participants) including overweight or obese individuals with metabolic disease found that probiotics reduced BMI (MD −0.73 kg/m^2, 95% CI −1.31 to −0.16, P = 0.01), waist circumference (MD −0.71 cm, 95% CI−1.24 to −0.19, P = 0.008), and hip circumference (MD −0.73 cm, −1.16 to −0.30, P = 0.0008) [33]. Based on this analysis, the strains Lactobacillus (*L. Casei* strain Shirota (LAB13), *L. Gasseri*, *L. Rhamnosus*, *L. Plantarum*) and Bifidobacterium (*B. Infantis*, *B. Longum*, and *B. Breve* B3) showed protective benefits in the context of obesity. Meanwhile, analyses in which many of the included studies were comprised of participants of normal weight, significant effects on anthropomorphic measures, such as BMI, were not seen [34]. In several studies BMI was found to be a modifier of effect size such that those classified by overweight or obese, possibly indicative of greater metabolic dysfunction, were more likely to show beneficial effects from probiotic use [35; 36].

Diabetes: PROBIOTICS MAY IMPROVE GLYCEMIC CONTROL

Numerous meta-analyses of clinical trials indicate that probiotics can improve glycemic control in the context of type 2 diabetes, although particular glycemic parameters affected vary across trials, and the magnitude of the effect sizes are generally small. In addition to effects on glucose homeostasis, most studies also showed effects on blood lipid profiles, particularly with respect to cholesterol levels, as well as a reduction in blood pressure. A meta-analysis of 38 RCTs (n=2086 participants) found that probiotics, prebiotics or synbiotics decreased fasting blood glucose (MD −0.58 mmol/l, 95% CI −0.86 to −0.30, P < 0.01; 36 studies) and insulinaemia (MD −10.51 pmol/l, 95% CI −16.68 to−4.33, P < 0.01; 22 studies) in diabetic patients [37]. A meta-analysis of 28 RCTs (n=1947 participants) found that probiotic use improved fasting blood glucose in both short duration (<12 weeks) (MD -2.99 mg/dL, 95% CI –23.55 to –2.42; P=0.016; 9 studies, n=428) and long duration (<12 weeks) RCTs (MD -2.99 mg/dL, 95% CI –5.84 to –

0.13, P=0.040; 12 studies, n=805) [38]. There was also a reduction in HbA1c (MD –0.16, 95% CI –0.30 to –0.02, P= 0.023). The effects were greatest in participants with fasting blood glucose levels >130 mg/dL. A meta-analysis of 32 RCTs (n=1737 participants) found that probiotics reduced HbA1c (MD − 0.33%, 95% CI − 0.53 to − 0.13, P = 0.001; 14 studies), fasting plasma glucose (MD − 16.52 mg/dL, 95% CI − 23.28 to − 9.76, P < 0.001; 24 studies), and fasting insulin levels (MD 1.40 µIU/mL, 95% CI − 2.52 to − 0.27, P = 0.015; 15 studies) [39]. Significant reductions in fasting plasma glucose, HbA1c, fasting insulin, and HOMA-IR were also found in a meta-analysis of 14 RCTs [40]. In a meta-analysis of 13 RCTs (n=818 participants), multi-strain probiotics were found to exert greater effects on glycemic parameters, and non-glycemic parameters, such as lipid profile and blood pressure [35]. Overall, multi-strain probiotics appear to be the most beneficial in their ability to improve metabolic parameters in the context of diabetes and other metabolic disorders.

The hypoglycemic effects are thought to stem from the immunomodulatory and oxidative stress reducing properties of probiotics. Additionally, some strains have been shown to modulate appetite regulating hormones, and to influence the production of glucagon-like peptide-1 (GLP-1) [40].

Hypertension: PROBIOTICS MAY MODESTLY REDUCE BLOOD PRESSURE

Probiotic use is associated with a reduction in blood pressure across a variety of clinical trials, however, the effect size is very modest, thus the clinical significance of the effects is likely to be low in the context of hypertension. The blood pressure lowering effect is likely mediated through the ability of the gut-brain axis to modulate parasympathetic activity via the vagus nerve, and the production of bacterial metabolites to influence angiotensin-converting enzyme (ACE) activity [41].

A systematic review of 5 meta-analyses (n= 2703 participants) indicates a modest effect for probiotics in hypertensive adults with or without diabetes, with a lowering of systolic blood pressure (SBP) from 3.10 to 5.04 mmHg, and a lowering of diastolic blood pressure (DBP) from 0.39 to 3.84 mmHg [42]. Effects were greatest in participants with blood pressure ≥ 130/85, and when higher dosages (≥ 10^{11} CFU) were used. Similar findings were reported in subsequent meta-analyses. A meta-analysis of 23 RCTs (n=2037 participants) found that probiotic use was associated with a lowering of SBP by − 3.05 mmHg (95%CI − 4.67 to − 1.44; P < 0.001) and DBP by − 1.51 mmHg (95% CI − 2.38 to − 0.65; P = 0.001) [43]. A meta-analysis of 14 RCTs (n=846 participants) showed a reduction in SBP of − 2.05 mmHg (95% CI − 3.87 to − 0.24, P = 0.03), with better effects in participants under age 60, when treatment was longer than four weeks, and when high dosages were used (≥ 2 × 10^{10} CFU/day) [44]. DBP was lowered by − 1.26 mmHg (95% CI − 2.51 to − 0.004, P = 0.047), again preferentially in those younger than 60. A meta-analysis of seven RCTs (n=653 participants) using *Lactobacillus plantarum* containing probiotics found that this

strain was associated with a lowering of SBP by -1.58 mmHg (95 % CI -3.05 to 0.11) and a lowering of DBP by -0.92 mmHg (95 % CI -1.49 to -0.35) [45]. This suggests that while probiotics containing Lactobacillus plantarum have an anti-hypertensive effect, they may not be the optimal probiotic for this indication. Since comparative analyses have not yet been conducted for other strains, the composition of the optimal probiotic is unclear.

Dyslipidemia: PROBIOTICS MAY LOWER SERUM CHOLESTEROL LEVELS

Probiotics have been shown to modulate the blood lipid profile in a range of cardiometabolic conditions. Although, probiotics tend to shift the profile towards a healthy state, there is variability across clinical trials regarding which blood lipids are altered depending on the population tested and the composition of the probiotic. Studies involving healthy populations have been mixed, suggesting that probiotics have a minimal impact on blood lipid levels in the absence of an underlying condition [5].

A meta-analysis of 39 RCTs (n=2237 participants) testing fermented milk-based probiotics found significant reductions in blood LDL-c (WMD −7.34 mg/dL, 95% CI −10.04 to −4.65, P < 0.001) and total cholesterol (WMD −8.30 mg/dL, 95% CI −11.42 to −5.18, P < 0.001) [46].The effects on LDL-c were most pronounced in studies using strains of Bifidobacterium (WMD −16.25 mg/dl, 95% CI −22.08 to −10.42; 3 studies), and in participants who were overweight. Probiotics containing a mixture of Lactobacilli and Bifidobacteria strains appeared to be the most effective in modulating levels of serum HDL-c and triacylglycerol. In a meta-analysis of 34 RCTs (n=2177 participants) involving populations at risk for cardiovascular disease, longer duration studies (> 1.5 months) and those using higher dose probiotics (> 1.0×10^9 CFU) were more likely to show benefit on cardiovascular parameters [47]. Probiotics were associated with reductions in total cholesterol (MD − 6.05 mg/dL, 95% CI − 8.49 to − 3.61; 24 studies), and LDL-c (− 8.77 mg/dL, 95% CI − 11.86 to − 5.69; 21 studies), as well as an increase in HDL-c (1.05 mg/dL, 95% CI − 0.33 to 2.43; 21 studies), with greater effects in patients with hypercholesterolemia. Probiotics were also found to reduce total cholesterol (SMD −0.23, 95% CI −0.3 to −0.10) and triglyceride levels (SMD −0.27, 95% CI −0.44 to −0.11) in dyslipidemic patients with type 2 diabetes, based on meta-analyses of 13 RCTs (n=937 and n=1133 participants, respectively) [9].

Preclinical studies suggest that probiotic strains can reduce cholesterol levels through several mechanisms. These include the incorporation of cholesterol into bacterial membranes, the inhibition of HMG-CoA, the rate-limiting step in cholesterol biosynthesis, and promoting the deconjugation of bile acids through the enzyme bile salt hydrolase (BSH) [32; 39]. The deconjugated bile salts are then excreted, which promotes the production of new bile salts from cholesterol, thereby decreasing

cholesterol levels. Lactobacillus and Bifidobacterium probiotic strains have been shown to have high BSH activity, which likely accounts for their cholesterol-lowering properties [32].

Kidney disease: PROBIOTICS MAY REDUCE INFLAMMATION AND OXIDATIVE STRESS

Clinical trials in chronic kidney disease suggest that while probiotics may not have a significant impact on kidney function, they may be modestly protective against some of the associated cardiometabolic complications. A meta-analysis of 13 RCTs (n=671 participants) testing probiotics, prebiotics, and/or synbiotics found a significant reduction in the inflammatory marker CRP (SMD −0.75, 95% CI −1.03 to −0.47; 10 studies), the oxidative stress marker MDA (SMD −1.06, 95% CI −1.59 to −0.52; 6 studies) in studies longer than 12 weeks, total cholesterol (SMD −0.33, 95% CI −0.52 to −0.13; 8 studies), and LDL-c (SMD −0.44, 95% CI −0.86 to −0.02; 6 studies) [36]. There were also increases in the antioxidant GSH (SMD, 0.44, 95% CI 0.25 to 0.65; 6 studies), total antioxidant capacity (TAC) (SMD 0.61, 95% CI 0.07 to 1.15; 5 studies), and HDL-c (SMD 0.45; 95% CI 0.03 to 0.87; 7 studies). A separate meta-analysis of 14 RCTs (n=584 participants) showed similar effects on MDA, TAC, and GSH, but did not find significant effects on LDL, HDL, or CRP [34]. This analysis also found a reduction in hs-CRP (SMD −0.52, 95% CI, −0.81 to −0.22; 8 studies) and measures of glucose homeostasis, including fasting blood glucose, HOMA-IR, and insulin. A meta-analysis of 16 RCTs (n=605 participants) focused on inflammatory cytokines also showed a reduction of serum CRP levels (WMD -12.29, 95% CI -16.41 to -8.16), but did not find a significant effect on IL-6 or TNFα levels for probiotic use [48]. In patients with diabetic nephropathy, a meta-analysis of four RCTs (n=220 participants) found that probiotic use reduced hs-CRP and MDA, while increasing TAC and GSH, with higher dosages (>5 billion CFU) associated with greater benefits [49]. Overall, these analyses suggest that probiotics can reduce inflammatory and oxidative stress, but the effects may vary depending on the type of kidney disease.

Osteoporosis: PROBIOTICS HAVE MINIMAL EFFECTS ON BONE DENSITY

Tested probiotics appear to have minimal effect on bone health. A meta-analysis of five RCTs (n=497 participants), found that probiotic use was associated with higher bone mineral density in the lumbar spine (SMD 0.27, 95% CI 0.09 to 0.44), but not in the hip (SMD 0.22, 95% CI −0.07 to 0.52) in postmenopausal women. Levels of collagen type 1 cross-linked C-telopeptide (CTX), a marker of bone reabsorption were reduced with probiotic treatment (SMD −0.34, 95% CI −0.60 to −0.09), but there were no significant effects on bone-specific alkaline phosphatase, osteoprotegerin, osteocalcin, or TNF-α [50]. A separate meta-analysis of eight RCTs (n=564 participants) found that while the use of probiotics, prebiotics, or synbiotics elevated serum calcium levels (0.52 mg/dl, 95% CI 0.38 to 0.66), there were no

significant effects on bone mineral density in the hip or spine, and no significant effects on levels of parathyroid hormone, osteocalcin, and alkaline phosphatase in middle-aged and older adults [51].

Infections and immune regulation: PROBIOTICS HAVE IMMUNOMODULATORY EFFECTS

Probiotics can modulate immune responses by regulating the composition and activity profile of bacterial species in the gut. Approximately 70% of the immune system is located in the gut, which means that alterations in the gut microbiota can have profound effects on systemic inflammatory and immune responses [52]. Metabolites produced by the microbiota can influence T cell function and cytokine production. Depending on the context, the microbiome can dampen inflammation or potentiate the immune response against pathogens.

The ability of probiotics to protect against infections has been studied in athletes, since excessive exercise can weaken the immune system. A meta-analysis of 14 RCTs (n=1309 participants) involving athletes found that probiotic supplementation reduced total symptom severity score for upper respiratory tract infections (URTI) (SMD –0.65, 95% CI –1.05 to –0.25, P = 0.02; 8 studies), but did not have significant effects on the mean number or duration of upper respiratory tract infection episodes [53]. Probiotic use was also associated with a reduction in levels of the inflammatory cytokines IL-6 (SMD –2.52 pg/ml, 95% CI –4.12 to –0.51, P = 0.001; 8 studies) and TNF-α (SMD –2.31 pg/ml, 95% CI –4.12 to –0.51, P = 0.008; 8 studies). A Cochrane meta-analysis of 12 RCTs (n=3720 participants) including children and adults found that probiotic use was associated with reduced numbers of participants experiencing acute URTI (Odds ratio [OR] 0.53, 95% CI 0.37 to 0.76, P< 0.001), a reduction in the mean duration of an episode of acute UTRI (MD -1.89, 95% CI -2.03 to -1.75, P < 0.001), and reduced antibiotic prescription rates for acute URTIs (OR 0.65, 95% CI 0.45 to 0.94) [54]. A separate meta-analysis of six RCTs (n=1551 participants) found that probiotics reduced the incidence of URTI episodes (Risk ratio [RR] 0.77, 95% CI 0.68 to 0.87, P < 0.0001) in adults [55]. Probiotics have been found to be marginally effective in boosting immunity against the common cold in healthy adults [56], as well as for influenza in the elderly [57]. A systematic review of three RCTs found that probiotics were protective against oral Candida fungal infections in the elderly, though the evidence is limited [58]. Efficacy may depend on the nature of the pathogen, but the immune environment of a given person is likely to play a larger role.

The immunomodulatory effects are generally modest, and it is not clear whether probiotics can have a significant immunostimulatory effect in immunosuppressed patients. A meta-analysis of 16 RCTs found that the use of probiotics, prebiotics, and synbiotics did not significantly affect CD4 T cell levels in patients with HIV [59]. The data are also inconclusive regarding whether probiotics can significantly enhance immune responses in the elderly. A meta-analysis of 15 RCTs (n= 5916 participants) testing

probiotics in older adults, with an average age of 75.21 years, found that probiotic use was not significantly associated with a reduction in the occurrence of infection, mean duration of infection, adverse events, or mortality [60]. The majority of these studies involved residents of long-term care facilities who may have been frail and/or in poor health. Meta-analyses of eight (n=1083 participants) and 14 RCTs (n=1975 participants) found that probiotic use was associated with a reduction in the incidence of ventilator-associated pneumonia, with odds ratios of 0.70 (95% CI 0.52 to 0.95) and 0.62 (95% CI 0.45 to 0.85), respectively, though there were no significant effects on reducing mortality in this population [61; 62]. These studies suggest that while probiotic strains can potentiate weak immune responses in individuals who are otherwise in good health, they act in the context of an individual's overall physiological state, and thus have limited capacity for immunomodulation in the context of a severely impaired or dysfunctional immune system.

Most analyses combine trials using different strains of probiotics, but a few have been done which provide evidence that particular strains consistently show immunomodulatory properties. Head-to-head comparisons are lacking, so it is unclear which strains have the best potential for immune stimulation or dampening. It is likely that different populations may preferentially benefit from different strains depending on their baseline immune state. A meta-analysis of 18 RCTs (n=1047 participants) using probiotics containing *Lactobacillus plantarum*, found that this strain has immunomodulatory properties. Its use was associated with reductions in IL-4 (MD −0.48 pg/mL, 95% CI −0.79 to −0.17; $P < 0.05$) and IFN-γ (MD −0.99 pg/mL, 95% CI −1.56 to −0.41; $P < 0.05$), as well as increases in the anti-inflammatory cytokine IL-10 (MD 9.88 pg/mL, 95% CI 6.52 to 13.2; $P < 0.05$) [63]. A meta-analysis of four clinical trials found that *Bifidobacterium animalis* ssp. lactis HN019 increased the phagocytic capacity of polymorphonuclear cells (SMD 0.74, 95% CI 0.38 to 1.11, $P < 0.001$) and the tumoricidal activity of NK cells (SMD 0.43, 95% CI 0.08 to 0.78, $P = 0.02$) in healthy elderly adults (median age 60 to 70) [64].

Safety: Probiotics are generally safe, but due to their activity in gut, may produce temporary discomfort in the gastrointestinal system. Probiotics-related infections are rare, but can occur in high-risk immunocompromised individuals.

Types of evidence:

- 6 meta-analyses of RCTs on safety of probiotics
- 2 agency reports
- 1 review on safety for probiotics

In 2011, the Agency for Healthcare Research and Quality (AHRQ) released a comprehensive report including 622 studies assessing the safety of probiotics. While they found that probiotic use was not associated with increased risk for adverse events (Relative risk [RR] 1.00; 95% CI 0.93 to 1.07, P=0.999), this was based solely on short-term clinical trial data [65]. Additionally, this finding is reported with low confidence due to the poor reporting of safety outcomes in probiotics trials. Numerous meta-analyses of clinical trials assessing the safety and efficacy of probiotics for a variety of different conditions have been conducted since that report, nearly all of which reach the same conclusion that probiotic use is safe and not associated with a greater incidence of adverse events relative to placebo, but that the supporting evidence is weak due to poor reporting [38; 55; 66; 67; 68].

The most common side effects with probiotic use are related to the gastrointestinal system, such as abdominal cramping, nausea, soft stools, flatulence, and taste disturbance [69]. This likely accounts for the mixed results in trials assessing probiotics for inflammatory bowel diseases [67; 70]. These effects are usually transient, and may be related to an individual's gut environment and microbiota. Probiotic bacterial species release bioactive metabolites which may have different effects in different people. For example, lactic bacteria produce metabolites which are inactivated by enzymes in the gut, but if those enzymes are absent or inactive, the metabolites can cause headaches [70].

The general consensus is that probiotics are safe in most people, but may increase the risk for infection in certain high-risk populations, such as immunocompromised patients, premature infants, patients with short bowel syndrome, patients with central venous catheters, and patients with cardiac valve disease [69]. There have been several case reports of fungemia and bacteremia in these populations, as well as cases of sepsis when the bacteria leak from the gut and enter the circulating blood [71]. Since the evidence for benefit is also often weaker, the therapeutic risk-benefit profile may not support the general use of probiotics in these high-risk populations.

In 2002 the WHO and FDA outlined four theoretical risks for probiotic use [1; 69]. Systemic infection, as described, has been documented in the context of immune suppression, but is generally a rare occurrence. Due to their production of bioactive metabolites, probiotic strains have the potential to influence metabolic activities, which under some conditions could be deleterious. In most cases, probiotic use is associated with the alleviation of certain aspects of metabolic disease, however, there have been some reported cases of disrupted metabolism in patients with short bowel syndrome. Due to its immunoregulatory activity, probiotics could potentially over activate the immune system and trigger an autoimmune reaction, but this has not yet been reported. The fourth concern refers to the risk of gene transfer, if probiotic strains become vectors for the transfer of antibiotic resistance genes.

Preclinical studies have indicated that this is a potential risk, but it has not been well examined in clinical studies, thus far.

Drug interactions: Antibiotics and antifungal drugs may interact with probiotics (WebMD). Other strain specific drug interactions are possible if the strains produce metabolites that interfere with the activity or metabolism of the drug. These effects may depend on host factors.

Sources and dosing:

Probiotics are available OTC, however, due to the lack of clear regulatory standards and oversight, the quality of OTC products is extremely variable [3]. Probiotics are available in supplements as well as in live culture containing fermented foods, such as yogurt. Lactobacillus and Bifidobacterium are the most common genera of bacteria used in clinically tested probiotics, but there are numerous different species, and each one has different properties. Probiotic strains are selected for based on their culturability and ability to remain viable during their journey through the digestive tract. As a result, the bacterial strains that have the most potent biological activity may not be the best candidates for probiotics and vice versa. Lactobacillus is known for its resistance to the acidic conditions of the stomach, which allows for eventual colonization of the gut [15]. Bifidobacterium has been shown to be able to elicit protective effects simply by passing through the gut [5]. The host diet plays a role in the potential efficacy of probiotics, as sufficient prebiotics (dietary fiber) are necessary to support the growth and colonization of the probiotic bacterial strains. Due to the role of the host gut environment, different people are likely to preferentially benefit from different probiotic strains [72]. No optimal strain or combination of strains has been established for any condition, as there have been few clinical trials evaluating different strains in a head-to-head manner. Ultimately, the optimal probiotic will be personalized to the individual.

The primary mechanisms by which probiotic strains exert their beneficial effects is thought to be through their enhancing gut barrier integrity and function, regulating immune cells, and producing bioactive metabolites, such as SCFAs [26]. Strains that act by improving gut barrier function may be best suited to disorders of the gastrointestinal tract. The profile of bioactive metabolites will impact the immuno-, neuro-, and metabolo-regulatory properties of a given probiotic, thus the assessment of this profile as part of clinical studies may facilitate the optimization of probiotic strains for different conditions. Due to host-related factors, the metabolite profile of the probiotics is likely to vary across individuals, thus biomarkers will be needed to guide the selection of efficacious strains for a given person.

Lactobacillus rhamnosus GG strain is one of the most clinically tested strains. *Lactobacillus rhamnosus* GG-containing probiotics have shown efficacy in a variety of indications. This strain also has a strong safety record based on Finnish surveillance data [69].

The dose of probiotic strains is measured in colony forming units (CFUs). No optimal dose has been established, as it depends on the strain and the indication [5]. Meta-analyses consistently show that preparations with higher CFUs, or on the order of five to ten billion CFU, tend to have higher efficacy in clinical trials [32; 42; 47]. The higher dose allows for a greater chance of productive colonization and survival in the gut. The optimal number of strains and dosing for multi-strain probiotics is unclear, because the strains may end up competing with one another, leading to different outcomes, depending on the conditions [16].

The optimal duration of treatment depends on the condition, and whether it is acute or chronic. With chronic conditions, longer duration treatment is often necessary to see benefit. For example, in the context of cognitive impairment, studies typically need to be at least 12 weeks long to see evidence of benefit [16]. Some analyses find that the benefits for a certain condition are greater in short duration trials, which may be indicative of compensatory effects, or could be related to the use of different strains across studies [39; 43]. The effects are generally related to the colonization or presence of the supplemented strains within the gut; thus, the effects tend to be limited to the period of probiotic treatment [5]. Some studies have found shifts in overall microbiota composition; however, no clear trends have emerged with respect to microbiome changes and efficacy [8; 32]. This suggests that supplementation with probiotic strains can alleviate some of the effects of gut microbiota dysbiosis, but on its own is unlikely to fix the underlying dysbiosis. For more lasting effects, probiotic supplementation needs to be coupled with dietary changes.

Research underway:

According to Clinicaltrials.gov, there are currently 404 active clinical trials involving probiotics for a wide range of conditions.

Search terms:

Pubmed, Google: Probiotics

- Alzheimer's disease, Parkinson's disease, cognition, cardiovascular, clinical trials, meta-analysis, systematic review, safety

Websites visited for Probiotics:

- Clinicaltrials.gov
- Examine.com
- Drugs.com
- WebMD.com
- Labdoor.com
- ConsumerLab.com
- Cafepharma

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