ROLE OF DIET, PHYSICAL ACTIVITY AND IMMUNE SYSTEM IN PARKINSON'S DISEASE

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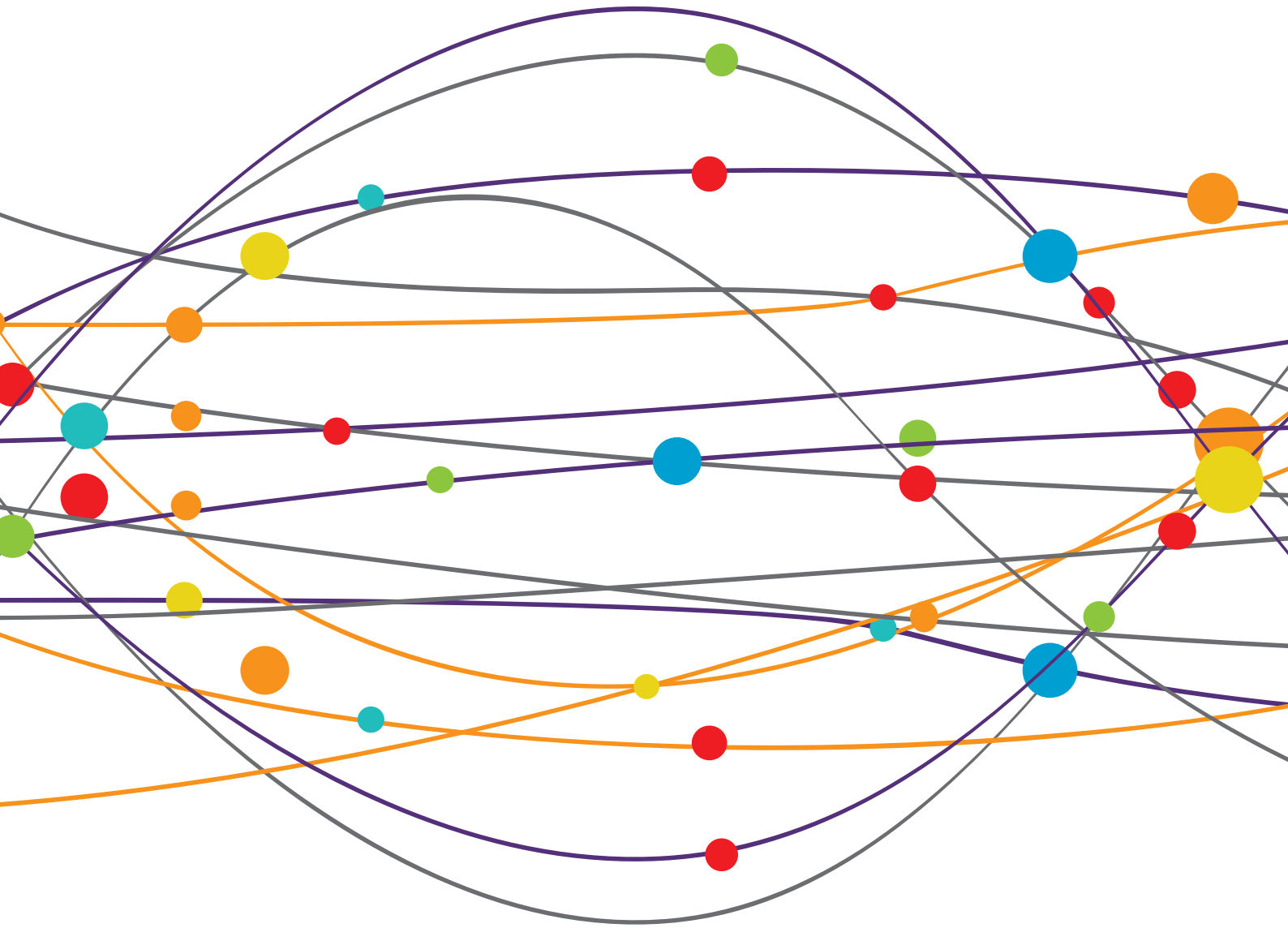

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ROLE OF DIET, PHYSICAL ACTIVITY AND IMMUNE SYSTEM IN PARKINSON'S DISEASE

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Editorial: Role of Diet, Physical Activity and Immune System in Parkinson's Disease

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Editorial on the Research Topic

Role of Diet, Physical Activity and Immune System in Parkinson's Disease

It is believed that Parkinson's disease (PD) may originate outside of the central nervous system and converging evidence strongly suggests that gastrointestinal tract (GI) tract may be a critical system in PD pathogenesis. **(a)** The GI tract is the largest surface area and point of entry for environmental factors to interact with the host. **(b)** The microbiota that inhabit the GI tract are profoundly impacted by environmental factors including those that are known to be risk factors for PD pathogenesis such as high fat, high sugar/low fiber Western diet, and lack of physical activity [Jackson et al., (1)]. **(c)** GI tract dysfunction such as constipation is commonly observed in PD patients and often occurs decades before PD diagnosis and an abnormal microbiota (i.e., dysbiosis) is observed with those with constipation (2). **(d)** A pathological hallmark of PD, α-synuclein misfolding and aggregation, is thought to be a consequence of inflammation and the source of that inflammation could be the microbiota. Indeed, the intestinal microbiota (especially a pro-inflammatory, dysbiotic microbiota) can activate intestinal mucosal, systemic, and brain immune systems, which can culminate in neuroinflammation (3). **(e)** PD patients have microbiota dysbiosis with low levels of anti-inflammatory short chain fatty acids (SCFA) and high levels of pro-inflammatory lipopolysaccharide (LPS) (4, 5). **(f)** The GI tract is continuously inundated with a high antigenic load resulting from exposure to pathogens, pathobionts, and commensal bacteria leading to chronic mucosal immune activation (6). A combination of pro-inflammatory dysbiotic microbiota and exaggerated mucosal immune activation due to intestinal leak in PD appears to be the underlying mechanism of intestinal inflammation in PD. Examination of colonic biopsy tissue from PD patients demonstrate high levels of pro-inflammatory cytokines (TNFα, IL-1β, IFNγ, IL-5) (7). Production of these cytokines is important because co-culture of autologous Th17 cells with dopaminergic neurons showed that Th17 cell production of IL-17A damage DA neurons resulting in cell death (8). GI tract inflammation is a feature associated with PD, even during early stages of the disease, and this mucosal immune activation/inflammation may trigger and/or sustain neuroinflammation is required for α-synuclein aggregation and loss of dopaminergic neurons in PD. Taken together, these findings provide compelling evidence to support the view that the dysbiotic intestinal microbiota is a trigger and/or enabler for the sustained neuroinflammation that can initiate and/or promote PD pathogenesis.

Among the lifestyle factors most strongly implicated in PD pathogenesis are diet and physical activity. Consumption of a Western diet is a risk factor for PD whereas diets high in fiber are associated with reduced risk. While diet has many effects on the body (e.g., omega-3-fatty acids, polyphenols), diet robustly impacts the intestinal microbiota. Consumption of a Western diet

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promotes pro-inflammatory intestinal microbiota dysbiosis, characterized by a high relative abundance of LPS-containing bacteria and low relative abundance of SCFA-producing bacteria (4), which can promote neuroinflammation. Additionally, there is a growing body of evidence that physical activity reduces the risk of developing PD (9) and ameliorates Parkinsonism symptoms (10–15), and quality of life (16). Imaging studies demonstrate that exercise is associated with greater release of DA in the ventral and dorsal striatum, increases neurotrophin levels, improves vascularization, facilitates synaptogenesis, reduces inflammation, and reduces disordered protein deposition (15). It is not clear how physical activity mediates these beneficial effects on PD patients, but it may include changes in the microbiota. For example, physical activity is associated with a reduction in pro-inflammatory LPS-containing bacteria and an increase in SCFA-producing bacteria. It is possible that both diet and physical activity via changes in the microbiota modulate neuroinflammation and PD pathogenesis via changes in immune function.

How does dysbiotic microbiota trigger/promote neuroinflammation? The microbiota has many features that can influence inflammation, but compelling evidence indicates that low SCFA and high LPS [via binding to toll like 4 receptor (TLR) leading to activation of the NLRP3 inflammasome] are important. SCFA are thought to be anti-inflammatory with an inverse relationship observed between SCFA levels and pro-inflammatory cytokines such as IL-6, IL-12, and TNF-α (17). SCFA can cross the blood brain barrier and microglia (resident immune cells in the brain) are influenced by SCFA (18). SCFA (especially butyrate) are essential for health of intestinal colonic epithelial cells (19) and low SCFA are associated with disrupted intestinal barrier with a concurrent increase in LPS in the systemic circulation (20). There is also a substantial amount of data demonstrating the importance of the LPS-induced NLRP3 inflammasome activation in PD (21). Activation of the NLRP3 inflammasome following exposure to microbial (e.g., LPS or damage-associated stimuli) induces robust inflammation (e.g., production of IL-1β). NLRP3 levels increase in response to factors such as consumption of a Western diet and it could be that this primes the immune system to respond to increased LPS (as is the case of microbiota dysbiosis) (22). These mechanisms (and others) might be the underlying mechanism for impact of the diet and physical activity on PD pathogenesis and disease course.

This special issue will present evidence demonstrating the critical role of the intestinal microbiota and the immune system in PD pathogenesis, as well as how diet and physical exercise might impact PD disease course (via a mechanism including an altered microbiota). This special issue highlights the potential importance of the bi-directional relationship of the brain and intestinal microbiota and microbiota/immune system in PD underscoring the need to better understand the microbiota-gut-brain axis in PD in order to identify potential therapeutic target(s) to design scientifically-based, gut/microbiota-directed disease modifying therapeutics to prevent and/or treat PD and positively impact PD disease course.

AUTHOR CONTRIBUTIONS

GA conceived the idea of this topic and managed most of the reviews. Consequently, he developed a first draft of this contribution, reporting the highlights of the topic, and gave specific aspects about physical activity. AK supervised this work and gave a significant contribution about the role of GA. SA supervised the contribution and offered specific aspects about immune system. All authors discussed the results and contributed to the final manuscript.

REFERENCES

1. Mailing LJ, Allen JM, Buford TW, Fields CJ, Woods JA. Exercise and the gut microbiome: a review of the evidence, potential mechanisms, and implications for human health. *Exerc Sport Sci Rev.* (2019) 47:75–85. doi: 10.1249/JES.0000000000000183
2. Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* (2016) 15:1257–72. doi: 10.1016/S1474-4422(16)30230-7
3. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. *Cell.* (2016) 167:1469–80.e12. doi: 10.1016/j.cell.2016.11.018
4. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord.* (2015) 30:1351–60. doi: 10.1002/mds.26307
5. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Bürmann J, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat Disord.* (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
6. Sun M, He C, Cong Y, Liu Z. Regulatory immune cells in regulation of intestinal inflammatory response to microbiota. *Mucosal Immunol.* (2015) 8:969–78. doi: 10.1038/mi.2015.49
7. Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T. Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977-2014. *Gut.* (2019) 68:18–24. doi: 10.1136/gutjnl-2017-315666
8. Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. *Cell Host Microbe.* (2015) 17:565–76. doi: 10.1016/j.chom.2015.04.011
9. Ahlskog JE. Aerobic exercise: evidence for a direct brain effect to slow parkinson disease progression. *Mayo Clin Proc.* (2018) 93:360–72. doi: 10.1016/j.mayocp.2017.12.015
10. Ridgel AL, Peacock CA, Fickes EJ, Kim CH. Active-assisted cycling improves tremor and bradykinesia in Parkinson's disease. *Arch Phys Med Rehabil.* (2012) 93:2049–54. doi: 10.1016/j.apmr.2012.05.015
11. Allen NE, Sherrington C, Paul SS, Canning CG. Balance and falls in Parkinson's disease: a meta-analysis of the effect of exercise and motor training. *Mov Disord.* (2011) 26:1605–15. doi: 10.1002/mds.23790
12. Hackney ME, Earhart GM. Effects of dance on gait and balance in Parkinson's disease: a comparison of partnered and nonpartnered dance movement. *Neurorehabil Neural Repair.* (2010) 24:384–92. doi: 10.1177/1545968309353329
13. Murray DK, Sacheli MA, Eng JJ, Stoessl AJ. The effects of exercise on cognition in Parkinson's disease: a systematic review. *Transl Neurodegener.* (2014) 3:5. doi: 10.1186/2047-9158-3-5

14. Abrantes AM, Friedman JH, Brown RA, Strong DR, Desaulniers J, Ing E, et al. Physical activity and neuropsychiatric symptoms of Parkinson disease. *J Geriatr Psychiatry Neurol.* (2012) 25:138–45. doi: 10.1177/0891988712455237
15. Intlekofer KA, Cotman CW. Exercise counteracts declining hippocampal function in aging and Alzheimer's disease. *Neurobiol Dis.* (2013) 57:47–55. doi: 10.1016/j.nbd.2012.06.011
16. Cruise KE, Bucks RS, Loftus AM, Newton RU, Pegoraro R, Thomas MG. Exercise and Parkinson's: benefits for cognition and quality of life. *Acta Neurol Scand.* (2011) 123:13–9. doi: 10.1111/j.1600-0404.2010.01338.x
17. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. *J Exp Med.* (2012) 209:975–86. doi: 10.1084/jem.20112457
18. Park M, Ross GW, Petrovitch H, White LR, Masaki KH, Nelson JS, et al. Consumption of milk and calcium in midlife and the future risk of Parkinson disease. *Neurology.* (2005) 64:1047–51. doi: 10.1212/01.WNL.0000154532.98495.BF
19. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol.* (2006) 40:235–43. doi: 10.1097/00004836-200603000-00015
20. Makki K, Deehan EC, Walter J, Bäckhed F. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe.* (2018) 23:705–15. doi: 10.1016/j.chom.2018.05.012
21. Gordon R, Albornoz EA, Christie DC, Langley MR, Kumar V, Mantovani S, et al. Inflammasome inhibition prevents alpha-synuclein pathology and dopaminergic neurodegeneration in mice. *Sci Transl Med.* (2018) 10:eaah4066. doi: 10.1126/scitranslmed.aah4066
22. Chakraborty S, Kaushik DK, Gupta M, Basu A. Inflammasome signaling at the heart of central nervous system pathology. *J Neurosci Res.* (2010) 88:1615–31. doi: 10.1002/jnr.22343

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Dietary Vitamin E as a Protective Factor for Parkinson's Disease: Clinical and Experimental Evidence

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Effective disease-modifying treatments are an urgent need for Parkinson's disease (PD). A putative successful strategy is to counteract oxidative stress, not only with synthetic compounds, but also with natural agents or dietary choices. Vitamin E, in particular, is a powerful antioxidant, commonly found in vegetables and other components of the diet. In this work, we performed a questionnaire based case-control study on 100 PD patients and 100 healthy controls. The analysis showed that a higher dietary intake of Vitamin E was inversely associated with PD occurrence independently from age and gender (OR = 1.022; 95% CI = 0.999–1.045; $p < 0.05$), though unrelated to clinical severity. Then, in order to provide a mechanistic explanation for such observation, we tested the effects of Vitamin E and other alimentary antioxidants *in vitro*, by utilizing the homozygous PTEN-induced kinase 1 knockout (*PINK1*$^{-/-}$) mouse model of PD. *PINK1*$^{-/-}$ mice exhibit peculiar alterations of synaptic plasticity at corticostriatal synapses, consisting in the loss of both long-term potentiation (LTP) and long-term depression (LTD), in the absence of overt neurodegeneration. Chronic administration of Vitamin E (alpha-tocopherol and the water-soluble analog trolox) fully restored corticostriatal synaptic plasticity in *PINK1*$^{-/-}$ mice, suggestive of a specific protective action. Vitamin E might indeed compensate PINK1 haploinsufficiency and mitochondrial impairment, reverting some central steps of the pathogenic process. Altogether, both clinical and experimental findings suggest that Vitamin E could be a potential, useful agent for PD patients. These data, although preliminary, may encourage future confirmatory trials.

Keywords: Parkinson's disease, Vitamin E, antioxidant, neuroprotection, protective factors, diet, PINK1, synaptic plasticity

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INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder, idiopathic, and multifactorial, mainly due to the loss of dopaminergic neurons in the *Substantia Nigra pars compacta* (SNpc) and to excessive brain accumulation of α-synuclein positive cytoplasmic Lewy bodies (LB). PD causes a progressive and disabling syndrome, including motor and non-motor disturbances, which severely impair patients' quality of life. Therefore, effective disease modifying treatments represent an unmet clinical need (1–3).

Successful neuroprotection may imply a combined approach against the different partners of neurodegeneration (4). Since oxidative stress is a major player of the neurodegenerative process in PD, the modulation of redox balance has been extensively explored as a potential strategy to prevent neural death and disease progression. Indeed, different antioxidant agents are under investigation in several clinical trials (5–9). Besides synthetic compounds, an invaluable source of natural antioxidants is food, namely fruits and vegetables (10). Therefore, an antioxidant-rich diet could represent a viable option to boost antioxidant pathways counteracting neurodegeneration.

Multiple antioxidant species can be found in food, which operate differently in cellular metabolism. Vitamin E, in particular, is a powerful antioxidant, found in plants and abundant in many aliments consumed in our diet (11). Vitamin E family includes a number of lipophilic molecules (α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols), whose antioxidant properties rely on lipoperoxyl radical scavenging activity (11). Their neuroprotective effects have been demonstrated in multiple experimental models; likewise, the reduced levels of these molecules in humans have been associated with the occurrence of neurodegenerative diseases (12, 13).

We hypothesized that a higher dietary intake of Vitamin E might protect from progressive neurodegeneration in PD. Therefore, we conducted a study including: (1) a retrospective assessment of dietary Vitamin E intake (VEI) in PD patients compared to healthy controls, aimed at determining if a different dietary VEI is associated with diverse clinical conditions; (2) an *in vitro* protocol in brain slices of a PD mouse model, aimed at evaluating the effects of Vitamin E on synaptic plasticity abnormalities, a peculiar endophenotype observed in distinct PD models. Specifically, we used homozygous PTEN-induced kinase 1 (*PINK1*) knockout mice ($PINK1^{-/-}$), an established model of subclinical PD, which might reflect the early phases of the disease. In this model, we previously observed a significant decrease in dopamine release, which is the major determinant of the loss of bidirectional synaptic plasticity at corticostriatal synapses. Indeed, both long-term potentiation (LTP) and long-term depression (LTD) are impaired in these mice, in the absence of overt neuronal degeneration, thereby representing an early pathophysiological event preceding cellular death (14–16).

METHODS

Case-Control Study

Population

The study involved 200 consecutive subjects (100 PD patients and 100 sex/age matched controls), afferent to the Neurology Unit of Tor Vergata University Hospital (Rome, Italy). PD was diagnosed according to the United Kingdom PD Society Brain Bank criteria. Controls (CTL) were healthy subjects, without history of neurological diseases or neurological signs at clinical examination, enrolled among non-blood relatives of patients. Exclusion criteria were cognitive decline with Mini-Mental status Examination (MMSE) <25 (adjusted for age and educational level); gastrointestinal disorders and malabsorption; abdominal surgery; diabetes; obesity (BMI > 29); alcoholism; internal failures (e.g., liver, heart); feeding problems; dietary restrictions; habit to taking vitamins integration. All the participants signed a written informed consent. The study was carried out according to the Declaration of Helsinki and was approved by the local ethical committee (Tor Vergata, Rome—Italy; number 98–09).

Assessment of VEI

All subjects underwent a structured *ad hoc* interview assessing dietary habits: the interview relied on a questionnaire, including explicative pictures to avoid misunderstanding. Subjects were asked how frequently each specific Vitamin E-rich aliment was habitually consumed in the preceding year (2 = more than once a week; 1 = at least once a month; 0 = never). Vitamin E-rich aliments' daily portions were named in a list including fresh fruits (e.g., kiwi, mango), dried fruits (e.g., almonds, walnuts), vegetable (e.g., spinach, broccoli), seeds (e.g., sunflower, pumpkin), oil (e.g., olive, sunflower), fish (e.g., bluefish, crayfish); (source: US Department of Agriculture, USDA (17)). Individual VEI was finally estimated by summing the products of each food's vitamin E content (mg) * the frequency of eating (0, 1, 2). Vitamin E content values were obtained from the Swedish Food Administration Database (18).

Statistical Analysis

The distribution of collected variables was preliminary examined with the Shapiro–Wilk test. Then, the non-normally distributed data were log-transformed to allow statistical analysis. Differences between the groups were tested by parametric (one-way ANOVA) or non-parametric (chi-square) tests, as appropriate. In addition, possible differences in the VEI depending on the H&Y stage and gender were tested by using the one-way ANOVA. The association between PD and VEI was assessed by means binomial logistic regression, adjusting the model for age and gender.

Experimental Electrophysiology on PD Mouse Model

Animal Model and Experimental Setting

Treatment and handling of animals were carried out in accordance with both the EC and Italian guidelines (86/609/EEC; DLS 116/1992, Directive 2010/63/EU; DLS/26 04/03/2014) and were further approved by the University of Rome Tor Vergata statute (n. 153/2001A) and by Animal Care and Use Committee of University of Rome "Tor Vergata." Transgenic mice (8- to 10-weeks old) were generated as previously described (14).

Intracellular recordings were obtained from striatal neurons in a parasagittal brain slice (300 μm) (9, 19, 20). A single slice was transferred in a recording chamber (35°C, 2–3 ml/min) and submerged in a continuously flowing Krebs' solution (35°C, 2–3 ml/min) bubbled with 95% O_2 and 5% CO_2. Kreb's solution was composed of (in mM): 126 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaC1_2$, 10 glucose, and 18 $NaHCO_3$. Intracellular recording electrodes were filled with 2 M KCl (30–60 MΩ). To evoke excitatory postsynaptic potentials (EPSPs), a bipolar electrode was placed in the white matter, in close proximity to the recording electrode or in layer VI of the cortex. Test stimuli were delivered at a frequency of 0.1 Hz in the presence

of 50 μM Picrotoxin to block GABA A-mediated responses. The pharmacological effects on EPSPs recorded from knockout mice (*PINK1*$^{-/-}$) were calculated as percentage of control amplitude in the wild-type (WT) or *PINK1*$^{+/+}$ neuronal population.

For high-frequency stimulation (HFS, three trains 100 Hz, 3 s, 20 s apart), stimulus intensity was raised to reach threshold level. After HFS delivery, the amplitude of EPSPs was plotted over-time as percentage of the control EPSP. Magnesium was omitted from the medium for LTP induction (9, 19, 20).

Signals were recorded with an Axoclamp 2B amplifier (Axon Instruments, Foster City CA 94404, USA), displayed on a oscilloscope and stored on PC using Digidata 1,500 A and pClamp 10.6 (Axon Instruments, Molecular Devices, USA). Data were examined of line by clampfit 10.7 software (Axon Instruments, Molecular Devices, USA). After initial analysis, all data were elaborated by Origin Microcal 2016 (Adalta) software.

Treatments

Effects of Vitamin E (alpha-tocopherol and the water-soluble analog Trolox) were assessed in comparison to other antioxidant agents of alimentary origin, in order to test their action specificity. In particular, for our experiments we specifically selected: beta-carotene (21, 22), lycopene (22, 23), lutein (24), folic acid (25), ascorbic acid/Vitamin C (26), retinol/Vitamin A (27), Vitamin K1/phylloquinone, and Vitamin K2/menaquinones (28, 29).

The effects of drug treatments in PINK$^{-/-}$ mice were tested in two different conditions: (1) *ex-vivo*, by acute preincubation of parasagittal brain slices; (2) *in vivo*, by chronically administered intraperitoneal injections.

For acute treatment, a single slice was incubated, from 40 min before HFS induction and for the duration of the whole experiment (about 1 h), in a bath solution containing the drug dissolved in Krebs' solution. Selected compounds were used in bath at the respective dose of: alpha-tocopherol = 100 μM and Trolox = 100 μM (9); beta-carotene = 100 μM (30); lutein = 20 μM (24); lycopene = 5 μM (22, 23); folic acid = 100 μM (25); Vitamin A = 1 μM (27); Vitamin C = 1–3 mM (26); Vitamin K1 = 20 μM, and Vitamin K2 = 10 μM (28, 29).

For chronic treatments, all compounds were solved in ringer lactate and administered via intraperitoneal injections for 7 days consecutively (9). Dose treatment was: alpha-tochoperol = 100 mg/kg/7 days and Trolox = 5 mg/kg/7 days (9); beta-carotene = 2 mg/kg/7 days (31); lutein = 3 mg/kg/7 days (32); lycopene = 50 mg /kg/7 days (31); folic acid = 2 mg/kg/7 days (33); Vitamin A = 0.5 mg/kg/7 days (31); Vitamin C = 100 mg/kg/7 days (34); Vitamin K2 = 50 mg/kg/7 days (35); Vitamin K1 = 150 mg/kg/7 days (36).

Drug Source

Beta-carotene, lycopene, folic acid, lutein, folic acid, Vitamin A, Vitamin C, Vitamin K1 and K2, alpha-tochopherol, and Trolox were purchased from Sigma-Adrich, Italy. All the other drugs were purchased by Panreac Quimica (Spain).

TABLE 1 | Clinical-demographic parameters and VEI values of the study population.

		PD		CTL		Significance
Gender	(M/F)	55.2%	44.8%	46.9%	53.1%	ns
Age (years)	Mean	63.3		60.5		
	St.dev.	8.5		10.6		
Log10 Age	Mean	1.80		1.77		ns
	St.dev.	0.06		0.08		
VEI (mg)	Mean	31.6		38.4		
	St.dev.	13.5		17.8		
Log10 VEI	Mean	1.45		1.54		$p < 0.05$
	St.dev.	0.21		0.21		
H&Y	Mean	2.4		–		
	St.dev.	0.7		–		

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical significance between pre and post HFS stimulation was evaluated using Student *T*-test. Percentage values were calculated for each individual experiment. An analysis of variance with the Tukey's *post-hoc* test was performed among the groups ($P < 0.05$; alpha = 0.01). Statistical significance was set at <0.05.

RESULTS

Case-Control Study

Clinical-demographic parameters and the VEI of the study population are summarized in **Table 1**. PD and CTL were homogeneous in age and gender distribution; VEI was significantly higher in CTL (38.4 mg ± 17.8) than PD (31.6 mg ± 13.5; Statistical analysis was conducted on Log-transformed values, resulting $p <$ 0.05). Conversely, VEI did not differ depending on the gender in both groups, neither among the stages of H&Y in PD patients. The binomial logistic regression showed that VEI was directly associated with CTL status, independently from age and gender (Odd Ratio, OR = 1.022; 95% CI = 0.999–1.045; $p < 0.05$).

Electrophysiology in PD Mouse Model

According to our previous findings (14, 16), HFS protocol performed on parasagittal slice preparation induced a robust LTD in MSNs recorded from WT mice (58.24 ± 3.79% of control; $n = 16$ **Figure 1A**), whereas it failed to elicit LTD in *PINK1*$^{-/-}$ mice (99.61 ± 2.88% of control; $n = 22$ $p < 0.05$ *t*-test **Figure 1A**). After removal of magnesium from the bathing medium, HFS induced LTP in WT mice (168.26 ± 5.21% of control; $n = 12$; *t*-test $p < 0.05$ **Figure 1B**). In *PINK1*$^{-/-}$ mice, HFS also increased EPSPs compared to pre HFS (123.39 ± 4.88% of control; $n = 12$; *t*-test $p < 0.05$, **Figure 1B**), but the magnitude was significantly lower than WT mice ($p < 0.05$ ANOVA), suggesting the impairment of this form of plasticity.

None of the other drugs, but Vitamin E, was able to rescue either LTD or LTP in both acute and chronic treatment

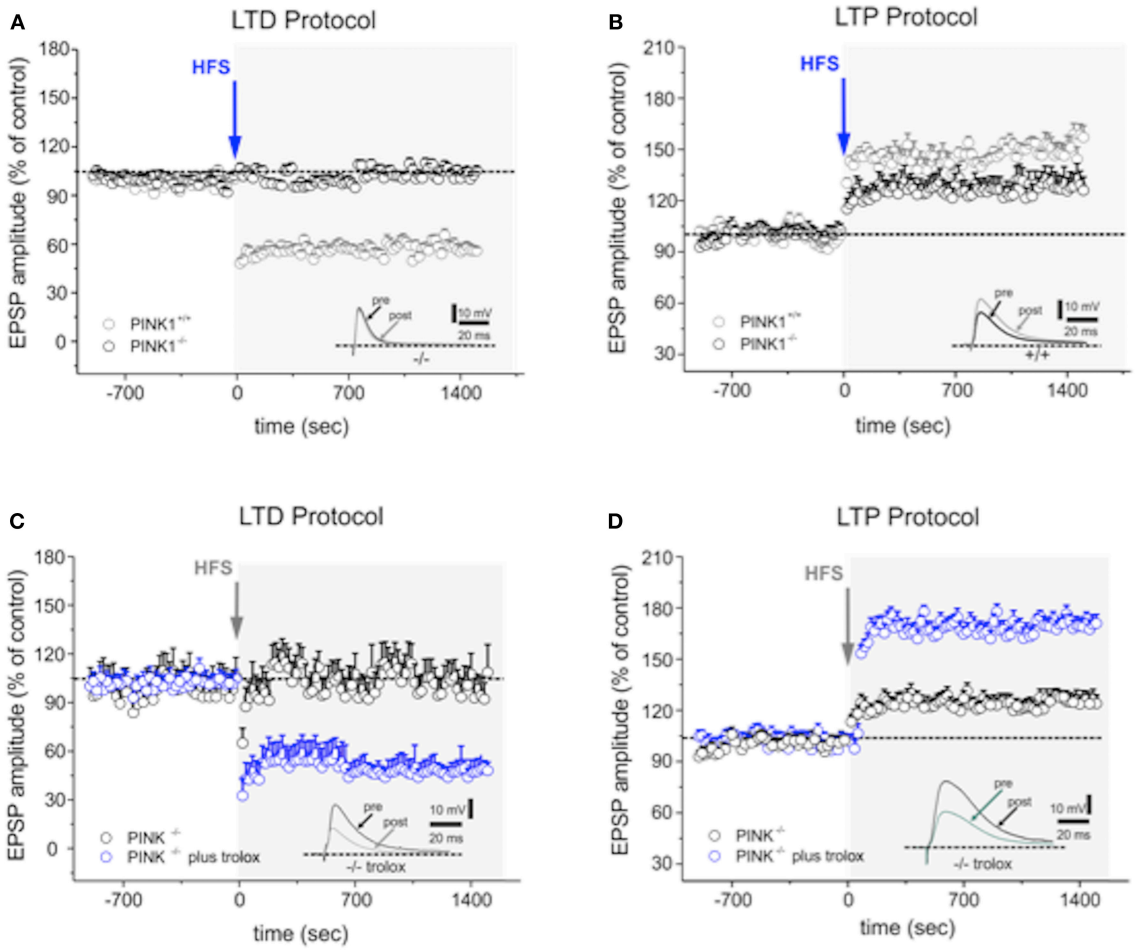

FIGURE 1 | Trolox fully rescues both forms of altered synaptic plasticity in *PINK1*$^{-/-}$ mice. **(A)** Time-course of LTD in WT and *PINK1*$^{-/-}$ mice recorded from parasagittal slices. HFS (arrow) induces LTD in WT mice (gray circles), but not in *PINK1*$^{-/-}$ mice (black circles). The inset shows a representative sample of EPSPs recorded in *PINK1*$^{-/-}$ before (pre) and 20 min after (post) HFS. **(B)** The LTP induction protocol causes LTP in WT mice but not in *PINK1*$^{-/-}$ mice (black circles). The magnitude of LTP measured in *PINK1*$^{-/-}$ mice is significantly reduced. The inset shows a representative sample of EPSPs recorded in *PINK1*$^{-/-}$ before (pre) and 20 min after (post) HFS. **(C,D)** In slices acutely treated with Trolox 100 μM (blue circles) or in slices from mice chronically treated with Trolox, both LTD **(C)** and LTP **(D)** is rescued. LTD is fully rescued, while LTP increases in magnitude as in normal condition. The insets show two representative samples of EPSPs recorded in *PINK1*$^{-/-}$ before (pre) and 20 min after (post) HFS. Each data point represents the mean ± SEM from acute and chronic treatment, respectively.

(>5 observations for each experimental condition; *T*-test, $p > 0.05$; **Table 2**). Specifically, Trolox (the water-soluble analog of Vitamin E) fully rescued both the forms of synaptic plasticity either after acute or chronic administration (LTD protocol: 55.88 ± 6.01% of control; $n = 16$; *t*-test $p < 0.05$ **Figure 1C**; LTP protocol: 175.30 ± 4.88% of control; $n = 10$; *t*-test $p < 0.05$ **Figure 1D**, **Table 2**); alpha-tochoperol restored in chronic conditions (intraperitoneal injection) (LTD protocol: 56.9 ± 4.21% of control; $n = 8$; *t*-test $p < 0.05$; LTP protocol: 177.2 ± 6.36% of control; $n = 6$; *t*-test $p < 0.05$, **Table 2**).

DISCUSSION

In this study, both the clinical retrospective analysis and our electrophysiological experiments demonstrate that Vitamin E might exert potential beneficial effects in PD.

The case-control analysis showed that dietary VEI is higher in healthy subjects than age/sex matched PD patients. Such a reduced intake in PD patients might suggest a lack of its putative protective action, independently from age and gender. Although consistent with other data from larger and prospective cohorts (17, 18), a number of limitations should be considered in the interpretation of the result, such as the sample size, the recall bias, and the absence of accurate measurement for the dietary intake. In fact, VEI was just approximately estimated by a retrospective *ad hoc* questionnaire, scoring how frequently the standard portions of aliments with Vitamin E higher content were assumed in the last year, and not precisely quantified. However, to prevent confounding factors due to the occurrence of the disease, we excluded from the study patients with alimentary restrictions (e.g., dysphagia), dementia or any concomitant condition affecting feeding behavior or

TABLE 2 | The table summarizes the effects of acute and chronic treatment of every single compound on corticostriatal synaptic plasticity.

Drug	Administration	Rescue LTD	Rescue LTP
Beta-carotene	Acute	NO	NO
	Chronic		
Lutein	Acute	NO	NO
	Chronic		
Lycopene	Acute	NO	NO
	Chronic		
Folic acid	Acute	NO	NO
	Chronic		
Vitamin A	Acute	NO	NO
	Chronic		
Vitamin C	Acute	NO	NO
	Chronic		
Vitamin K2	Acute	NO	NO
	Chronic		
Vitamin K1	Acute	NO	NO
	Chronic		
Alpha-tocopherol (Vitamin E)	Acute	NO	NO
	Chronic	**YES**	**YES**
Trolox (vitamin E)	Acute	**YES**	**YES**
	Chronic	**YES**	**YES**

Only alpha-tocopherol and Trolox resulted effective. Bold highlights drugs restoring synaptic plasticity.

intestinal absorption. Moreover, the homogeneous distribution of demographic features in the study population and the statistical methodology might have limited the influence of other potential confounding factors (e.g., sex/age-based dietary choices, sex/age–dependent differences in internal metabolism). In addition to confirm previous findings (17, 18), here we noticed that VEI did not correlate with severity of PD, assessed by H&Y score. Actually, the sample size and the exclusion from the model of other clinical determinants (e.g., disease duration, therapy) might represent a bias; indeed, it is possible that both levodopa dose and disease duration, which are usually higher in more advanced patients, may affect vitamin absorption (37), causing some deficiency unrelated to the alimentary habits. Also the occurrence and severity of constipation, which in turn might influence dietary choices, pharmacotherapy and intestinal function (38), has not been addressed in the study. Therefore, caution is required in the interpretation of our preliminary results. To this regard, it should be mentioned that other authors, also by utilizing recall-based questionnaires, excluded significant associations between alimentary VEI and PD (39, 40); these studies differed in sample size, but were performed out of Mediterranean area. Hence, we should consider regional diet as a further potential confounding factor. Certainly, prospective cohort studies, eventually supported by direct vitamin dosage, are necessary to assess the weight of VEI in PD pathogenesis.

The protective action of Vitamin E on PD has been further explored by using an experimental model of preclinical PD. *PINK1*$^{-/-}$ mice indeed exhibit the disruption of bidirectional plasticity at corticostriatal synapses, even in the absence of overt neurodegeneration. Several studies indicate this model as representative of a critical time-window of the disease in which a specific intervention may revert the pathophysiological cascade leading to symptoms onset, being thus appropriate to test the efficacy of disease-modifying strategies (3, 14–16). Our experiments show that the administration of Vitamin E (alpha-tocopherol and Trolox), but not other dietary antioxidant compounds (beta-carotene, lycopene, lutein, folic acid, ascorbic acid/Vitamin C, retinol/Vitamin A, Vitamin K1/phylloquinone, and Vitamin K2/menaquinones), was able to revert synaptic plasticity abnormalities in *PINK1*$^{-/-}$ mice. *PINK1* haploinsufficiency precipitates mitochondrial functioning, impairing mitophagy, and above all, energy production under increased demand (41). This, in turn, accounts for the reduced synaptic vesicle release at dopaminergic terminals and the subsequent breakdown of corticostriatal synaptic plasticity (9, 14, 16). It is thus conceivable that Vitamin E, unlike other vitamins, specifically rescues striatal homeostasis and neurotransmission in *PINK1*$^{-/-}$ mice, by enhancing mitochondrial metabolism (42, 43) and energy-dependent processes. Indeed, it has been recently demonstrated that Vitamin E, but not other antioxidants, such as Vitamin C, fully rescued longevity in a short-lived *Candida elegans gas-1(fc21)* model of respiratory chain complex I defect (44). Moreover, in other experimental models, Vitamin E resulted to be able to activate cellular pathways involved in antioxidant, detoxifying, and anti-inflammatory responses and to promote bioenergy at mitochondrial level (45, 46). Regarding *PINK1*$^{-/-}$ rodents model, Shim and colleagues demonstrated that Trolox dramatically improved mitochondrial metabolism in PINK1-deficient dopaminergic cells, by increasing complex I and complex IV's activity (47). Because neurotransmitter release depends on mitochondrial bioenergetics (48), we hypothesize a recovery of a physiological dopaminergic transmission with the subsequent rescue of corticostriatal plasticity. However, a specific set of experiments is required to assess this issue in *PINK1*$^{-/-}$ mice. Yet, the complex interactions between PINK1-mediated mitochondrial activities and Vitamin E-induced cellular reactions (49, 50) could then explain the inefficacy of other proved alimentary antioxidants in restoring corticostriatal synaptic plasticity.

Since mitochondrial dysfunction is critical also in pathogenesis of idiopathic PD (41, 51, 52), such a mechanism may justify a beneficial action of higher dietary VEI on PD in humans. Furthermore, Vitamin E seems to intervene on other pathogenic pathways of PD, such as lysosome metabolism (53), expanding potential restorative effects. Definitely, larger studies are mandatory to validate this hypothesis.

Regardless the limitations, our findings suggest a potential protective action of a Vitamin E rich diet. These data may indicate that Vitamin E represents a potential therapeutic target for disease-modifying treatments in PD. Therefore, diets including Vitamin E rich aliments could be an immediate option to reduce the risk of PD and other neurodegenerative

diseases (54), although specific confirmatory trials are necessary.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

TS, GM, and AP conceived the study and wrote the manuscript. GD, VC, DF, and MA collected clinical data. GM and PI performed the experiments. TS and MP performed statistical analysis. NM, MP, and PS contributed to interpretation of results and edited the manuscript.

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REFERENCES

1. Schirinzi T, Martella G, Elia AD, Di Lazzaro G, Imbriani P, Madeo G, et al. Outlining a population (at risk) of Parkinson' s disease : evidence from a case-control study. *Parkinsons Dis.* (2016) 2016:9646057. doi: 10.1155/2016/9646057
2. Kalia LV, Lang AE. Parkinson's disease. *Lancet* (2015) 386:896–912. doi: 10.1016/S0140-6736(14)61393-3
3. Schirinzi T, Madeo G, Martella G, Maltese M, Picconi B, Calabresi P, et al. Early synaptic dysfunction in Parkinson's disease: insights from animal models. *Mov Disord.* (2016) 31:802–13. doi: 10.1002/mds.26620
4. Lang AE, Espay AJ. Disease modification in Parkinson's disease: current approaches, challenges, and future considerations. *Mov Disord.* (2018) 33:660–77. doi: 10.1002/mds.27360
5. Jin H, Kanthasamy A, Ghosh A, Anantharam V, Kalyanaraman B, Kanthasamy AG. Mitochondria-targeted antioxidants for treatment of Parkinson's disease: preclinical and clinical outcomes. *Biochim Biophys Acta Mol Basis Dis.* (2014) 1842:1282–94. doi: 10.1016/j.bbadis.2013.09.007
6. Surmeier DJ, Obeso JA, Halliday GM. Parkinson's disease is not simply a prion disorder. *J Neurosci.* (2017) 37:9799–807. doi: 10.1523/JNEUROSCI.1787-16.2017
7. Crotty GF, Ascherio A, Schwarzschild MA. Targeting urate to reduce oxidative stress in Parkinson disease. *Exp Neurol.* (2017) 298(Pt B):210–24. doi: 10.1016/j.expneurol.2017.06.017
8. Imbriani P, Schirinzi T, Meringolo M, Mercuri NB, Pisani A. Centrality of early synaptopathy in Parkinson's disease. *Front Neurol.* (2018) 9:103. doi: 10.3389/fneur.2018.00103
9. Martella G, Madeo G, Maltese M, Vanni V, Puglisi F, Ferraro E, et al. Neurobiology of Disease Exposure to low-dose rotenone precipitates synaptic plasticity alterations in PINK1 heterozygous knockout mice. *Neurobiol Dis.* (2016) 91:21–36. doi: 10.1016/j.nbd.2015.12.020
10. Thapa A, Carroll NJ. Molecular sciences dietary modulation of oxidative stress in Alzheimer's disease. *Int J Mol Sci.* (2017) 18:1583. doi: 10.3390/ijms18071583
11. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med.* (2014) 72:76–90. doi: 10.1016/j.freeradbiomed.2014.03.035
12. Kaneai N, Arai M, Takatsu H, Fukui K, Urano S. Vitamin E inhibits oxidative stress-induced denaturation of nerve terminal proteins involved in neurotransmission. *J Alzheimer's Dis.* (2012) 28:183–9. doi: 10.3233/JAD-2011-111133
13. Ulatowski LM, Manor D. Vitamin E and neurodegeneration. *Neurobiol Dis.* (2015) 84:78–83. doi: 10.1016/j.nbd.2015.04.002
14. Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, et al. Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. *Proc Natl Acad Sci USA.* (2007) 104:11441–6. doi: 10.1073/pnas.0702717104
15. Imbriani P, Sciamanna G, Santoro M, Schirinzi T, Pisani A. Promising rodent models in Parkinson's disease. *Parkinsonism Relat Disord.* (2018) 46:S10–4. doi: 10.1016/j.parkreldis.2017.07.027
16. Madeo G, Schirinzi T, Martella G, Latagliata EC, Puglisi F, Shen J, et al. PINK1 heterozygous mutations induce subtle alterations in dopamine-dependent synaptic plasticity. *Mov Disord.* (2014) 29:41–53. doi: 10.1002/mds.25724
17. Zhang SM, Hernán MA, Chen H, Spiegelman D, Willett WC, Ascherio A. Intakes of vitamins E and C, carotenoids, vitamin supplements, and PD risk. *Neurology* (2002) 59:1161–9.
18. Yang F, Wolk A, Håkansson N, Pedersen NL, Wirdefeldt K. Dietary antioxidants and risk of Parkinson's disease in two population-based cohorts. *Mov Disord.* (2017) 32:1631–6. doi: 10.1002/mds.27120
19. Martella G, Tassone A, Sciamanna G, Platania P, Cuomo D, Viscomi MT, et al. Impairment of bidirectional synaptic plasticity in the striatum of a mouse model of DYT1 dystonia: role of endogenous acetylcholine. *Brain* (2009) 132:2336–49. doi: 10.1093/brain/awp194
20. Maltese M, Stanic J, Tassone A, Sciamanna G, Ponterio G, Vanni V, et al. Early structural and functional plasticity alterations in a susceptibility period of DYT1 dystonia mouse striatum. *Elife* (2018) 7:33331. doi: 10.7554/eLife.33331
21. Chang C-S, Chang C-L, Lai G-H. Reactive oxygen species scavenging activities in a chemiluminescence model and neuroprotection in rat pheochromocytoma cells by astaxanthin, beta-carotene, and canthaxanthin. *Kaohsiung J Med Sci.* (2013) 29:412–21. doi: 10.1016/j.kjms.2012.12.002
22. Di Tomo P, Canali R, Ciavardelli D, Di Silvestre S, De Marco A, Giardinelli A, et al. β-Carotene and lycopene affect endothelial response to TNF-α reducing nitro-oxidative stress and interaction with monocytes. *Mol Nutr Food Res.* (2012) 56:217–27. doi: 10.1002/mnfr.201100500
23. Qu M, Jiang Z, Liao Y, Song Z, Nan X. Lycopene prevents amyloid [Beta]-induced mitochondrial oxidative stress and dysfunctions in cultured rat cortical neurons. *Neurochem Res.* (2016) 41:1354–64. doi: 10.1007/s11064-016-1837-9
24. Li S-Y, Lo ACY. Lutein protects RGC-5 cells against hypoxia and oxidative stress. *Int J Mol Sci.* (2010) 11:2109–17. doi: 10.3390/ijms11052109
25. Zhu M, Li B, Ma X, Huang C, Wu R, Zhu W, et al. Folic acid protected neural cells against aluminum-maltolate-induced apoptosis by preventing miR-19 downregulation. *Neurochem Res.* (2016) 41:2110–8. doi: 10.1007/s11064-016-1926-9
26. Harrison FE, May JM. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. *Free Radic Biol Med.* (2009) 46:719–30. doi: 10.1016/j.freeradbiomed.2008.12.018
27. Kona SL, Shrestha A, Yi X, Joseph S, Barona HM, Martinez-Ceballos E. RARβ2-dependent signaling represses neuronal differentiation in mouse ES cells. *Differentiation* (2017) 98:55–61. doi: 10.1016/j.diff.2017.11.002
28. Sakaue M, Mori N, Okazaki M, Kadowaki E, Kaneko T, Hemmi N, et al. Vitamin K has the potential to protect neurons from methylmercury-induced cell death *in vitro*. *J Neurosci Res.* (2011) 89:1052–8. doi: 10.1002/jnr.22630

29. Nakajima M, Furukawa S, Hayashi K, Yamada A, Kawashima T, Hayashi Y. Age-dependent survival-promoting activity of vitamin K on cultured CNS neurons. *Brain Res Dev Brain Res.* (1993) 73:17–23.
30. Lin H-W, Chang T-J, Yang D-J, Chen Y-C, Wang M, Chang Y-Y. Regulation of virus-induced inflammatory response by β-carotene in RAW264.7 cells. *Food Chem.* (2012) 134:2169–75. doi: 10.1016/j.foodchem.2012.04.024
31. Glise D, Riondel J, Favier A. Comparative distribution of beta-carotene and lycopene after intraperitoneal administration in mice. *In Vivo* (1998) 12:447–54.
32. Li S-Y, Yang D, Fu ZJ, Woo T, Wong D, Lo ACY. Lutein enhances survival and reduces neuronal damage in a mouse model of ischemic stroke. *Neurobiol Dis.* (2012) 45:624–32. doi: 10.1016/j.nbd.2011.10.008
33. Gupta A, Puri V, Sharma R, Puri S. Folic acid induces acute renal failure (ARF) by enhancing renal prooxidant state. *Exp Toxicol Pathol.* (2012) 64:225–32. doi: 10.1016/j.etp.2010.08.010
34. Sahraei H, Aliabadi AA, Zarrindast M-R, Ghoshooni H, Nasiri A, Barzegari-Sorkheh AA, et al. Ascorbic acid antagonizes nicotine-induced place preference and behavioral sensitization in mice. *Eur J Pharmacol.* (2007) 560:42–8. doi: 10.1016/j.ejphar.2006.12.019
35. Onodera K, Shinoda H, Zushida K, Taki K, Kamei J. Antinociceptive effect induced by intraperitoneal administration of vitamin K2 (menatetrenone) in ICR mice. *Life Sci.* (2000) 68:91–7.
36. Sakamoto N, Kimura M, Hiraike H, Itokawa Y. Changes of phylloquinone and menaquinone-4 concentrations in rat liver after oral, intravenous and intraperitoneal administration. *Int J Vitam Nutr Res.* (1996) 66:322–8.
37. Rajabally YA, Martey J. Levodopa, vitamins, ageing and the neuropathy of Parkinson's disease. *J Neurol.* (2013) 260:2844–8. doi: 10.1007/s00415-013-7079-8
38. Barichella M, Cereda E, Cassani E, Pinelli G, Iorio L, Ferri V, et al. Dietary habits and neurological features of Parkinson's disease patients: implications for practice. *Clin Nutr.* (2017) 36:1054–61. doi: 10.1016/j.clnu.2016.06.020
39. Hughes KC, Gao X, Kim IY, Rimm EB, Wang M, Weisskopf MG, et al. Intake of antioxidant vitamins and risk of Parkinson's disease. *Mov Disord.* (2016) 31:1909–14. doi: 10.1002/mds.26819
40. Morens DM, Grandinetti A, Waslien CI, Park CB, Ross GW, White LR. Case-control study of idiopathic Parkinson's disease and dietary vitamin E intake. *Neurology* (1996) 46:1270–4.
41. Pickrell AM, Youle RJ. The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron* (2015) 85:257–73. doi: 10.1016/j.neuron.2014.12.007
42. Duan L, Li J, Ma P, Yang X, Xu S. Vitamin E antagonizes ozone-induced asthma exacerbation in Balb/c mice through the Nrf2 pathway. *Food Chem Toxicol.* (2017) 107:47–56. doi: 10.1016/J.FCT.2017.06.025
43. Vineetha RC, Binu P, Arathi P, Nair RH. L-ascorbic acid and α-tocopherol attenuate arsenic trioxide-induced toxicity in H9c2 cardiomyocytes by the activation of Nrf2 and Bcl2 transcription factors. *Toxicol Mech Methods* (2018) 28:353–60. doi: 10.1080/15376516.2017.1422578
44. Polyak E, Ostrovsky J, Peng M, Dingley SD, Tsukikawa M, Kwon YJ, et al. N -acetylcysteine and vitamin E rescue animal longevity and cellular oxidative stress in pre-clinical models of mitochondrial complex I disease. *Mol Genet Metab.* (2018) 123:449–62. doi: 10.1016/j.ymgme.2018.02.013
45. Petrillo S, Piermarini E, Pastore A, Vasco G, Schirinzi T, Carrozzo R, et al. Nrf2-inducers counteract neurodegeneration in frataxin-silenced motor neurons: disclosing new therapeutic targets for friedreich's ataxia. *Int J Mol Sci.* (2017) 18:2173. doi: 10.3390/ijms18102173
46. Dinkova-Kostova AT, Abramov AY. The emerging role of Nrf2 in mitochondrial function. *Free Radic Biol Med.* (2015) 88:179–88. doi: 10.1016/j.freeradbiomed.2015.04.036
47. Shim JH, Yoon SH, Kim K-H, Han JY, Ha J-Y, Hyun DH, et al. The antioxidant Trolox helps recovery from the familial Parkinson's disease-specific mitochondrial deficits caused by PINK1- and DJ-1-deficiency in dopaminergic neuronal cells. *Mitochondrion* (2011) 11:707–15. doi: 10.1016/j.mito.2011.05.013
48. Madeo G, Martella G, Schirinzi T, Ponterio G, Shen J, Bonsi P, et al. Aberrant striatal synaptic plasticity in monogenic parkinsonisms. *Neuroscience* (2012) 211:126–35. doi: 10.1016/j.neuroscience.2011.07.065
49. Murata H, Takamatsu H, Liu S, Kataoka K, Huh N, Sakaguchi M. NRF2 regulates PINK1 expression under oxidative stress conditions. *PLoS ONE* (2015) 10:e0142438. doi: 10.1371/journal.pone.0142438
50. Buendia I, Michalska P, Navarro E, Gameiro I, Egea J, León R. Nrf2-ARE pathway: an emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. *Pharmacol Ther.* (2016) 157:84–104. doi: 10.1016/j.pharmthera.2015.11.003
51. Tufekci KU, Civi Bayin E, Genc S, Genc K. The Nrf2/are pathway: a promising target to counteract mitochondrial dysfunction in Parkinson's disease. *Parkinsons Dis.* (2011) 2011:314082. doi: 10.4061/2011/314082
52. Holmström KM, Kostov RV, Dinkova-Kostova AT. The multifaceted role of Nrf2 in mitochondrial function. *Curr Opin Toxicol.* (2017) 2:80–91. doi: 10.1016/j.cotox.2016.10.002
53. Marín T, Contreras P, Castro J, Chamorro D, Balboa E, Bosch-Morató M, et al. Vitamin E dietary supplementation improves neurological symptoms and decreases c-Abl/p73 activation in niemann-pick C mice. *Nutrients* (2014) 6:3000–17. doi: 10.3390/nu6083000
54. Barnard ND, Bush AI, Ceccarelli A, Cooper J, de Jager CA, Erickson KI, et al. Dietary and lifestyle guidelines for the prevention of Alzheimer's disease. *Neurobiol Aging* (2014) 35:S74–8. doi: 10.1016/j.neurobiolaging.2014.03.033

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More Research Is Needed on Lifestyle Behaviors That Influence Progression of Parkinson's Disease

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The variability of symptoms in Parkinson's disease (PD) suggests the need for individualized treatment. A key aspect of precision medicine is lifestyle risk factor modification, known to be important in the prevention and management of chronic illness including other neurological diseases. Diet, cognitive training, exercise, and social engagement affect brain health and quality of life, but little is known of the influence of lifestyle on PD progression. Given disease heterogeneity, absence of objective outcome measures, and the confounding effects of medication, investigating lifestyle as a potential therapy in PD is challenging. This article highlights some of these challenges in the design of lifestyle studies in PD, and suggests a more coordinated international effort is required, including ongoing longitudinal observational studies. In combination with pharmaceutical treatments, healthy lifestyle behaviors may slow the progression of PD, empower patients, and reduce disease burden. For optimal care of people with PD, it is important to close this gap in current knowledge and discover whether such associations exist.

Keywords: Parkinson's disease, lifestyle behaviors, observational studies as topic, longitudinal studies, multimodal treatment concept

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INTRODUCTION

Parkinson's disease (PD) is an age-related complex progressive neurodegenerative disorder, with key pathological features being the presence of alpha-synuclein-containing Lewy bodies and a loss of dopaminergic neurons in the substantia nigra (1). Years to decades preceding diagnosis, symptoms can include constipation, sleep behavior disorder, hyposmia, and anxiety (2). At diagnosis, hallmark motor symptoms of bradykinesia, as well as either resting tremor or rigidity, are defining (3).

The spectrum of motor and non-motor symptoms, and their impact on patient quality of life, suggests a need to individualize treatment. Current treatments primarily act to replace or boost existing dopamine, managing mostly motor symptoms. However, their long-term use leads to side effects, and reduced efficacy (4). Treatment of non-motor symptoms, including fatigue and cognitive impairment, is often secondary though they can have a significant impact on daily living (5, 6).

A broader range of therapeutic alternatives is needed to manage symptoms and ideally slow PD progression. The difficulty in therapeutic discovery is partially attributed to limited understanding

of PD pathogenesis, assuming similar disease mechanisms across clinically heterogeneous patients, and the absence of biological markers to measure disease progression (7, 8). Nevertheless, as the spectrum of individual symptoms is increasingly being recognized, precision medicine is receiving warranted attention.

A key aspect of precision medicine is attention to modifiable lifestyle risk factors, including nutrition and exercise, known to be important to neuronal health (9–11), and potentially important in secondary prevention of progression of PD. Several studies have shown associations between modifiable lifestyle factors and PD risk and outcomes (**Figure 1**). Reduced risk of developing PD is associated with physical activity and perversely with smoking, while increased risk is associated with constipation and anxiety or depression (12). Mind-body practices and endurance exercise can improve PD health outcomes (13, 14), however their long-term effects on neuroprotection or disease-modifying potential in PD remain inconclusive (4, 12, 15). Similarly, despite associations observed between PD risk and urate, dairy, and caffeine, the effects of nutrition on progression remain unclear (15–17). Further research is required to elucidate the long-term effects of lifestyle behaviors on PD management and progression if secondary prevention of PD with lifestyle modification is to be a realistic treatment option.

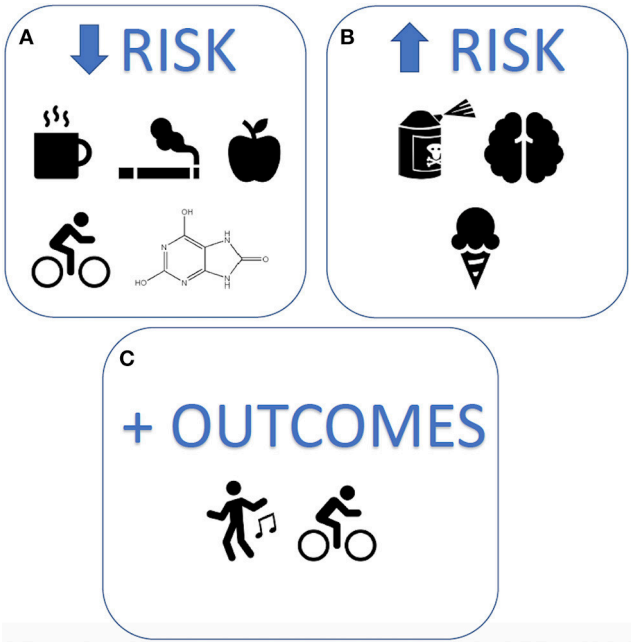

FIGURE 1 | Modifiable lifestyle factors associated with Parkinson's disease risk and outcomes. The strongest lifestyle factors associated with Parkinson's disease, reported to date, include **(A)** reduced risk: caffeine, smoking, uric acid, quality diets, and exercise **(B)** increased risk: exposure to pesticides, head injury, and dairy products, and **(C)** improved outcomes: mind-body exercises and physical activity.

STUDY DESIGNS TO MEASURE LIFESTYLE BEHAVIORS

Randomized controlled trials (RCTs) are the gold standard to examine therapeutic efficacy of an intervention (18, 19). However, selection bias, randomization, adherence, and short study duration often make RCTs impractical for lifestyle studies. In any event, there is scant information on which lifestyle factors might even be tested in such studies. To discover potential lifestyle exposures that might benefit neuronal health in PD and warrant trialing, unbiased monitoring of a population for lengthy periods is required. Here, registries can provide a valuable tool.

Barriers in establishing population-based registries include recruitment, cost, and data quality. While opt-out enrolment avoids recruitment bias, registries require close to 100% capture of patients with the disease in a given demographic. Extraction of data elements from patient electronic health records can save cost and time, with better data quality. Successful registries require significant collaborative efforts from clinicians and trained staff, to contribute data to a centralized repository. Time-poor clinicians may be reluctant to participate, and issues of data access, ownership, and governance can be additional barriers.

A cost- and time-efficient approach is an embedded trial within an existing database (18, 19). With this approach, a database with high quality data is required. Most existing databases capture predominantly Caucasian participants, recruit from hospitals, have low incident cases of PD, and collect little data on lifestyle behaviors (20). These issues could be lessened by combining comparable multi-center international cohorts and adding lifestyle variables to datasets. The success of combining cohorts necessitates a commitment to collaboration, standardized data definitions, data management and governance, and significant ongoing funding.

Observational cohort studies are less resource intensive than RCTs, and useful for complex study protocols and small patient populations (18). Selection bias and participant drop-out may be addressed through multifactorial recruitment and active engagement methods such as free access to wellbeing classes, and regular communication through newsletters, public seminars, and interactive workshops. Information bias and confounding may be minimized by design and analysis (19). In addition to efficiency, benefits of observational studies include minimal participant effort and adherence issues, as one follows natural behaviors.

Given that someone may follow more than one aspect of a healthy lifestyle, observational studies are most practical to evaluate associations of lifestyle and health outcomes. A proposed research design would be a longitudinal cohort study, with inclusion of an enriched PD population, caputuring data via a combination of data linkage to diagnosing and treatment clinics as well as self-reported online surveys (**Figure 2**). Selecting appropriate data variables to capture requires scientific rationale, with consideration of feasibility, practicality, and cost-effectiveness. The ability of potential recommendations to be seamlessly incorporated into people's everyday lives also needs to have a bearing on data capture.

Registry and observational studies can provide informed decisions for areas of focus for RCTs (18). Ideally, any strong

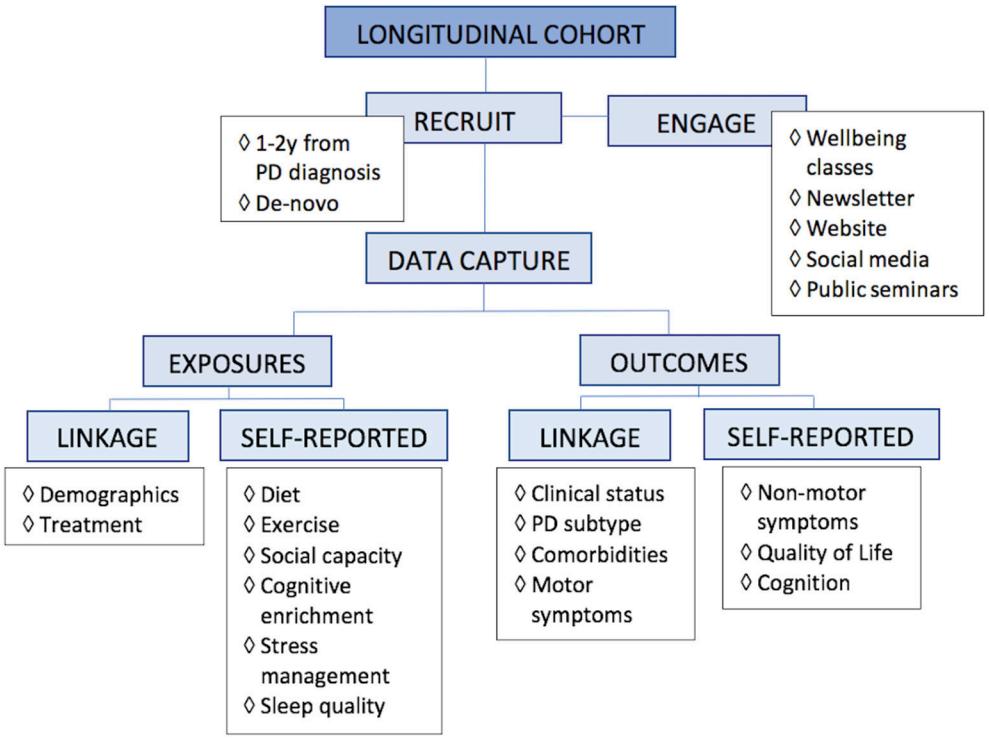

FIGURE 2 | Proposed research design for lifestyle. For research into multimodal lifestyle factors that impact Parkinson's disease, we propose a longitudinal study of an enriched population, capturing data via linkage, and self-reported online surveys.

association should be verified with a RCT prior to clinical recommendation. However, where common sense points to beneficial effects of low-risk modifiable behaviors on stress reduction, weight management, and cognitive engagement, health professionals may choose to prioritize patient education to incorporate healthy lifestyle into daily living.

LIMITATIONS OF EXISTING LIFESTYLE STUDIES IN PD

Several databases capture data on aging community members, as well as people at high risk of or diagnosed with PD. A 2017 study reviewed 44 of 68 identified PD databases around the world, showing that many include few incident cases of PD, little data on lifestyle, and were of limited duration (20). The authors highlight an unaddressed opportunity to combine these databases, thereby increasing research collaboration and knowledge of PD with a larger patient cohort.

Variability of interventions, improper controls, lack of relevant outcomes measures, and recruitment bias, make results of existing studies difficult to interpret or generalize (4). Additionally, there is no distinction or stratification of participants based on PD stage or subtype, which delineate disease symptoms and rate of progression (21, 22).

Questions remain unanswered on minimal dose requirements, distinction of a learnt response, sustainable effects once intervention ceases, as well as the impact of aging, baseline health, and comorbidities. The significant lack of evidence points to the need for an ongoing large-scale database to capture and monitor lifestyle and health outcomes in people with PD.

CHALLENGES OF LIFESTYLE OBSERVATIONAL STUDIES

Selection bias, confounding, and recruitment are key challenges. Multifactorial recruitment strategies and appropriate analysis can minimize selection bias and confounders, respectively (19). Screening for an enriched cohort may increase recruitment efficiency and the possibility of observing a therapeutic effect. Prodromal cohorts allow identification of PD in its earliest stages, with time to conversion being a measure of disease progression. Algorithms based on a combination of risk factors group participants into high, medium, and low risk of conversion, thereby potentially isolating an enriched, trial-ready population (23). Interventions are likely to have the most effect on this high-risk group as neurodegeneration is less established. Primary limitations are identifying participants with prodromal features, lack of generalizability given a selective PD sub-type, slow conversion of up to 14 years, and distinguishing intervention effects from slow rate of conversion.

Within diagnosed groups, extensive neuronal damage may result in barely perceptible effects of lifestyle changes, and these

may only affect non-motor symptoms. *De novo* participants with both short prodromal phase and time from diagnosis are favorable subjects, however misdiagnosis is common in this early phase (24). Most patients will be medicated within 12 months of diagnosis, after which time the effects of interventions are difficult to untangle. Measures of disease progression in diagnosed cohorts may therefore need to include time to pharmaceutical treatment, stable medication dose, motor or cognitive decline, and neuroimaging.

Study duration and participant retention are additional challenges. Lifestyle signals may be modest; therefore, an observational plan needs to be made for at least 5 years to see meaningful progression of the disease. Research funding is typically granted for 2–3 years, limiting potential for such data collection. To encourage retention, researchers should engage with participants by regularly communicating study milestones and other relevant and useful information, as well as promote involvement in events. Creative reminders and motivators to complete surveys with accuracy, to ensure unbiased data collection and analysis, are also important.

OUTCOME MEASURES

Lifestyle interventions are hard to measure precisely and may produce very specific and subtle signal changes. High baseline levels of healthy living are likely to be neuroprotective, thus increasing these levels may produce little change in health outcomes (25). Each intervention component should be measured at baseline and adjusted for effect size. Ideally, this would be measured with a combination of physiological markers and clinical assessments.

The development of markers of PD risk, diagnosis, and progression is a priority. Advances have been made for potential risk and diagnostic markers, including smell and sleep tests, imaging to detect dopamine neurotransmitter, alpha-synuclein, in the peripheral nervous system or cerebrospinal fluid, and gene variants in family members. As yet, no biomarker has however been validated as reliable or replicable for clinical use and none exists to measure disease progression (26). While important to provide insight into potential mechanisms for effective intervention, physiological tests often are not translatable to a clinically measurable outcome with which the patient can identify. Until sensitive and specific biomarkers are available to measure progression, a composite panel of clinical assessments is most appropriate.

Clinical assessments are recommended by the Movement Disorder Society (MDS) and a standard set of outcome measures recommended by the International Consortium for Health Outcomes Measurement (27). The MDS Unified Parkinson's Disease Risk Score [MDS-UPDRS; (28)] is the standard clinical measure for PD diagnosis and progression, though limited in detection of subtle improvements and susceptible to dopaminergic treatment effects and assessor subjectivity. Together, clinical measures of motor and non-motor symptoms, and quality of life, provide outcomes with relevance to the patient. These may be complemented with wearable devices and smart-phone applications that monitor PD specific behaviors (29). These technologies have the capacity to objectively measure changes in behaviors, including detailed information about patterns of movement, sleep quality, and blood pressure, with potential to develop computer programs to predict early indicators of PD, disease progression, and response to treatment. Determining which measures to assess requires consideration of data reliability and patient burden.

SUMMARY

Lifestyle has an important impact on risk and secondary prevention of many chronic conditions. There is increasing interest in the collection of lifestyle variables in PD cohorts. However, inadequate and lengthy self-reported recall surveys, the unlikelihood of lifestyle to have short-term or disease-modifying effects, and absence of objective outcome measures, are deterrents to capturing these data.

Given the complexity of symptoms in PD, the most viable therapeutic approach of lifestyle management may be multimodal. A combination of cognitive training, exercise, stress reduction, nutrition, and social components may be beneficial to quality of life. Whether these have a clinically significant effect on more objective health outcomes is best initially evaluated through longitudinal observational studies.

While there is much evidence on the benefits of lifestyle on general health outcomes (9, 10), such advice for people with PD must await a more concerted research effort to identify risk factors for disease progression. Then, implementation will require positive health promotion by health professionals, government, media, and policy makers. Health promotion initiatives can include prescribed exercise regimes, nutritional labels on foods, responsible marketing of tobacco and alcohol, and prioritizing wellbeing in educational and workplace organizations. While inducing long-term behavioral change is obviously a challenge, currently there is insufficient evidence to embark on such public health approaches in PD for most lifestyle factors, with the exception of exercise.

To enable a true overview of patient health and expedite research answers, data sharing and contribution to registries should be encouraged, and governments should prioritize resources for electronic data linkage between health services and research centers. The discovery of an evidence base around potential lifestyle modification in secondary prevention of PD progression depends on a much more robust and coordinated research effort world-wide than we have seen to date.

Modification of lifestyle risk factors is a foundational approach to prevention and management of chronic disease. These low-risk, self-managed therapies can empower the patient and reduce disease burden. Despite a robust evidence base in neurological diseases like stroke (10), there has been little coordinated effort

to discover such evidence in PD. Considering the growing burden of PD, this is an important omission in modern PD research and needs to be addressed.

AUTHOR CONTRIBUTIONS

NN: conception, organization, execution, writing, editing and final approval of the manuscript, accountability for the work. GJ: conception, critical manuscript revision and final approval, accountability for the work.

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REFERENCES

1. Braak H, Del Tredici K, Rub U, De Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
2. Pont-Sunyer C, Hotter A, Gaig C, Seppi K, Compta Y, Katzenschlager R, et al. The onset of nonmotor symptoms in Parkinson's disease (the ONSET PD study). *Mov Disord.* (2015) 30:229–37. doi: 10.1002/mds.26077
3. Berg D, Adler CH, Bloem BR, Chan P, Gasser T, Goetz CG, et al. Movement disorder society criteria for clinically established early Parkinson's disease. *Mov Disord.* (2018) 33:1643–46. doi: 10.1002/mds.27431
4. Fox SH, Katzenschlager R, Lim SY, Barton B, De Bie RMA, Seppi K, et al. International Parkinson and movement disorder society evidence-based medicine review: update on treatments for the motor symptoms of Parkinson's disease. *Mov Disord.* (2018) 33:1248–66. doi: 10.1002/mds.27372
5. Martinez-Martin P, Rodriguez-Blazquez C, Kurtis MM, Chaudhuri KR, Group NV. The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. *Movement Disord.* (2011) 26:399–406. doi: 10.1002/mds.23462
6. Duncan GW, Khoo TK, Yarnall AJ, O'brien JT, Coleman SY, Brooks DJ, et al. Health-related quality of life in early Parkinson's disease: the impact of nonmotor symptoms. *Mov Disord.* (2014) 29:195–202. doi: 10.1002/mds.25664
7. Charvin D, Medori R, Hauser RA, Rascol O. Therapeutic strategies for Parkinson disease: beyond dopaminergic drugs. *Nat Rev Drug Discov.* (2018) 17:804–22. doi: 10.1038/nrd.2018.136
8. Lang AE, Espay AJ. Disease modification in parkinson's disease: current approaches, challenges, and future considerations. *Mov Disord.* (2018) 33:660–77. doi: 10.1002/mds.27360
9. Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, et al. Dementia prevention, intervention, and care. *Lancet.* (2017) 390:2673–734. doi: 10.1016/S0140-6736(17)31363-6
10. Pandian JD, Gall SL, Kate MP, Silva GS, Akinyemi RO, Ovbiagele BI, et al. Prevention of stroke: a global perspective. *Lancet.* (2018) 392:1269–78. doi: 10.1016/S0140-6736(18)31269-8
11. Scarmeas N, Anastasiou CA, Yannakoulia M. Nutrition and prevention of cognitive impairment. *Lancet Neurol.* (2018) 17:1006–15. doi: 10.1016/S1474-4422(18)30338-7
12. Bellou V, Belbasis L, Tzoulaki I, Evangelou E, Ioannidis JP. Environmental risk factors and Parkinson's disease: an umbrella review of meta-analyses. *Parkinsonism Related Disord.* (2016) 23:1–9. doi: 10.1016/j.parkreldis.2015.12.008
13. Kwok JY, Choi KC, Chan HY. Effects of mind-body exercises on the physiological and psychosocial well-being of individuals with Parkinson's disease: a systematic review and meta-analysis. *Complement Ther Med.* (2016) 29:121–31. doi: 10.1016/j.ctim.2016.09.016
14. Flach A, Jaegers L, Krieger M, Bixler E, Kelly P, Weiss EP, et al. Endurance exercise improves function in individuals with Parkinson's disease: a meta-analysis. *Neurosci Lett.* (2017) 659:115–9. doi: 10.1016/j.neulet.2017.08.076
15. Bega D, Gonzalez-Latapi P, Zadikoff C, Simuni T. A review of the clinical evidence for complementary and alternative therapies in Parkinson's disease. *Curr Treat Options Neurol.* (2014) 16:314. doi: 10.1007/s11940-014-0314-5
16. Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* (2016) 15:1257–72. doi: 10.1016/S1474-4422(16)30230-7
17. Mischley LK, Lau RC, Bennett RD. Role of Diet and Nutritional Supplements in Parkinson's Disease Progression. *Oxid Med Cell Longev.* (2017) 2017:6405278. doi: 10.1155/2017/6405278
18. James S, Rao SV, Granger CB. Registry-based randomized clinical trials–a new clinical trial paradigm. *Nat Rev Cardiol.* (2015) 12:312–6. doi: 10.1038/nrcardio.2015.33
19. De La Torre Hernandez JM, Edelman ER. From nonclinical research to clinical trials and patient-registries: challenges and opportunities in biomedical research. *Rev Esp Cardiol.* (2017) 70:1121–33. doi: 10.1016/j.rec.2017.07.008
20. Heinzel S, Lerche S, Maetzler W, Berg D. Global, yet incomplete overview of cohort studies in Parkinson's disease. *J Parkinsons Dis.* (2017) 7:423–32. doi: 10.3233/JPD-171100
21. Aleksovski D, Miljkovic D, Bravi D, Antonini A. Disease progression in Parkinson subtypes: the PPMI dataset. *Neurol Sci.* (2018) 39:1971–6. doi: 10.1007/s10072-018-3522-z
22. Konno T, Deutschlander A, Heckman MG, Ossi M, Vargas ER, Strongosky AJ, et al. Comparison of clinical features among Parkinson's disease subtypes: a large retrospective study in a single center. *J Neurol Sci.* (2018) 386:39–45. doi: 10.1016/j.jns.2018.01.013
23. Noyce AJ, R'bibo L, Peress L, Bestwick JP, Adams-Carr KL, Mencacci NE, et al. PREDICT-PD: An online approach to prospectively identify risk indicators of Parkinson's disease. *Mov Disord.* (2017) 32:219–26. doi: 10.1002/mds.26898
24. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: a systematic review and meta-analysis. *Neurology.* (2016) 86:566–76. doi: 10.1212/WNL.0000000000002350
25. Rafferty MR, Schmidt PN, Luo ST, Li K, Marras C, Davis TL, et al. Regular exercise, quality of life, and mobility in Parkinson's disease: a longitudinal analysis of national parkinson foundation quality improvement initiative data. *J Parkinsons Dis Print.* (2017) 7:193–202. doi: 10.3233/JPD-160912
26. Gwinn K, David KK, Swanson-Fischer C, Albin R, Hillaire-Clarke CS, Sieber BA, et al. Parkinson's disease biomarkers: perspective from the NINDS Parkinson's Disease Biomarkers Program. *Biomark Med.* (2017) 11:451–73. doi: 10.2217/bmm-2016-0370
27. De Roos P, Bloem BR, Kelley TA, Antonini A, Dodel R, Hagell P, et al. A Consensus Set of Outcomes for Parkinson's Disease from the International Consortium for Health Outcomes Measurement. *J Parkinsons Dis.* (2017) 7:533–43. doi: 10.3233/JPD-161055
28. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord.* (2008) 23:2129–70. doi: 10.1002/mds.22340
29. Espay AJ, Bonato P, Nahab FB, Maetzler W, Dean JM, Klucken J, et al. Technology in Parkinson's disease: challenges and opportunities. *Mov Disord.* (2016) 31:1272–82. doi: 10.1002/mds.26642

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Potential of Prebiotic Butyrogenic Fibers in Parkinson's Disease

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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra. Recent evidence supports the involvement of the gastrointestinal tract in PD pathogenesis, including alterations in microbiota and intestinal permeability. Apart from being the preferred energy source for colonic epithelial cells, butyrate is involved in anti-inflammatory, enteroendocrine and epigenetic mechanisms that influence colonic and systemic health, including brain function. A few studies using oral administration of sodium butyrate indicate beneficial effects in PD animal models; however, prebiotic fibers that generate butyrate locally in the gut may be more effective. The design and selection of butyrogenic prebiotic fibers would allow preclinical studies to evaluate how gut-derived butyrate could affect PD pathophysiology. This review describes potential benefits of increasing gut butyrate production in PD through a prebiotic approach. Moreover, physico-chemical features of prebiotic fibers that target butyrogenic colonic bacteria are discussed.

Keywords: dietary fiber, Parkinson's disease, butyrate, gut microbiota, prebiotics

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INTRODUCTION

Parkinson's disease (PD) is a relentlessly progressive neurodegenerative disease of aging, with a considerable burden of disability. It is believed that PD pathology is a consequence of both genetic susceptibility and toxic environmental factors, resulting in increasing neuronal oxidative stress (1). The pathological hallmark of PD is neuronal inclusions termed Lewy bodies (LB) or Lewy neurites (LN) whose main component is aggregated and phosphorylated α-synuclein and is responsible for neurological symptoms and signs of PD (2).

Gastrointestinal involvement in PD may be pathogenic or a consequence of the disease. More recently, researchers have provided evidence that supports a role for the gastrointestinal tract and the enteric nervous system (ENS) in the pathogenesis of PD (3, 4). α-Synuclein aggregates are present in Substance P containing neurons in the sigmoid colonic submucosal neurons in patients with PD (5). Microbiota differs between those with PD and healthy controls; for instance, those with PD have a lower abundance of *Clostridium* cluster XIVa and IV (6–10). Changes in caecum mucosal-associated and luminal microbiota, including a significant decrease in the relative abundance of the beneficial commensal bacteria genus *Bifidobacterium*, has been induced by a mouse model of PD (11). Recently, evidence for proinflammatory dysbiosis in PD patients has been shown, and researchers suggest that this dysbiosis could trigger inflammation-induced misfolding of α-Syn and development of PD pathology (6). Additionally, intestinal permeability was increased and beneficial metabolites of microbiota function, such as short chain fatty acids (SCFA), were lower in those with PD compared to healthy controls (5). As evidence

for gastrointestinal tract involvement in PD exists, this suggests that therapeutic interventions may be warranted that positively impact the intestinal milieu by changing microbiota to produce less pro-inflammatory/injurious products and/or prevent gut leakiness.

PREBIOTIC FIBER: DEFINITION, STRUCTURE AND FUNCTION

The term prebiotics was first introduced in 1995 by Gibson and Roberfroid as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (12). Since then, the original definition has been revised several times and recently broadened to 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' (13).This should not be confused with probiotics, defined as "live microorganisms that confer a health benefit on the host when administered in adequate amounts" (14).

Although prebiotic definitions are general to all oligo- and polysaccharide prebiotic substrates, researchers up to 2010 have largely focused only on the use of fructans (fructooligosaccharides [FOS] and inulin), galactooligosaccharides (GOS) and, to a minor extent, lactulose, to promote beneficial shifts in the gut bacterial community (15). Prebiotic oligosaccharides were mainly used to promote increases in *Lactobacillus* and *Bifidobacterium* species (16). More recently, however, as the complexity and function of gut microbial ecosystems have been unveiled, new microbial groups or species of health interest have been identified, as well as ways to promote them (17–19). The challenge of achieving prebiotic effects favoring specific microbial groups requires the understanding of how prebiotic structure relates to substrate requirements of target bacteria and how they compete on substrates relative to other microbial groups (20).

The majority of prebiotic substrates fall into the dietary fiber classification—i.e., carbohydrate polymers not hydrolyzed by endogenous enzymes in the small intestine (21). Carbohydrates are the most abundant and heterogeneous class of molecules found in nature. In plants, non-cellulosic carbohydrate fibers include β-glucans, fructans, mannans, xylans, galactans, arabinans, arabinogalactans, pectins, and resistant starch. Also, carbohydrate fibers such as agars, sulfated carbohydrates, alginates, fucoidans, α,β-glucans and chitin may be found in other natural sources (22, 23). Apart from being a highly diverse class of molecules, complex variations at the fine chemical structure level (e.g., polymer size, linkage type, composition and arrangement of side chains, degree, and identity of ester-linked molecules) are possible within polymer class, resulting in dietary fibers with distinct solubilization degree, viscosity and tridimensional structure (20). For the complete hydrolysis and utilization of such complex molecules, a given gut bacteria should have within its genome the ability to produce recognition and binding proteins, transporters and carbohydrate-active enzymes (CAZymes) specific to a particular physicochemical structure (24). As such, the ability and efficiency in utilizing carbohydrates widely varies within gut individual bacteria or bacterial groups (24, 25). In addition, overlapping abilities in fiber degradation within bacterial species result in competitive pressures within the gut. For instance, Xu et al. (26) showed that strains of *B. cellulosilyticus* and *B. ovatus* both had the ability to grow on simple arabinoxylan structures. However, when the strains were cultivated together, *B. ovatus* outcompeted and dominated over *B. cellulosilyticus*. Thus, prebiotic fibers with specific physicochemical features can be selected to promote certain bacteria based on the ability of a bacteria or bacterial group to access and utilize them efficiently in the competitive environment of the colon (20).

METABOLITES FROM COLONIC DIETARY FIBER FERMENTATION IN PARKINSON'S DISEASE

The colonic fermentation of dietary fiber by specialist microbes in the gut leads to the formation of a variety of gases and metabolites. SCFAs including acetate, propionate, and butyrate comprise 90–95% of all microbiota metabolites produced in the colon (27–29). SCFAs hold biological significance and may act both locally in the gut and systemically to promote health benefits at distinct body sites. In neurological disorders, SCFAs are potentially important for their role in anti-inflammatory processes (30–32), promotion of blood-tissue barrier integrity (33, 34), and neuromodulation (35, 36). Moreover, local effects such as triggering gut peristaltic reflexes (37) could be relevant, as constipation is an usual clinical finding in many neurological disorders, including PD (38, 39). Although there are no studies evaluating acetate and propionate singly in PD, butyrate has been studied and the majority of preclinical evidence suggests that it specifically could be beneficial in many aspects of PD (40–45).

Butyrate

Butyrate is the preferred energy source for gut enterocytes, responsible for most of their energy metabolism (46). Butyrate also supports gut barrier function through the stimulation of tight junction assemblies and mucus production. As mentioned, hyperpermeability of the colonic epithelium occurs in PD (5); thus, the action of butyrate on the gut barrier may have clinical importance in PD. At the cell surface level, butyrate elicits a variety of physiological responses through G protein-coupled receptors (GPCR) in enterocytes (47). In particular, butyrate regulates inflammatory pathways that are important in maintaining gut homeostasis (48, 49) and stimulates the production of enteroendocrine hormones such as glucagon-like peptide 1 (GLP-1) and peptide YY (50, 51) (**Figure 1**). Both of these hormones reach circulation and exert their action through receptors spread at distinct body sites, including the brain. In a mouse model of PD, oral administration of sodium butyrate

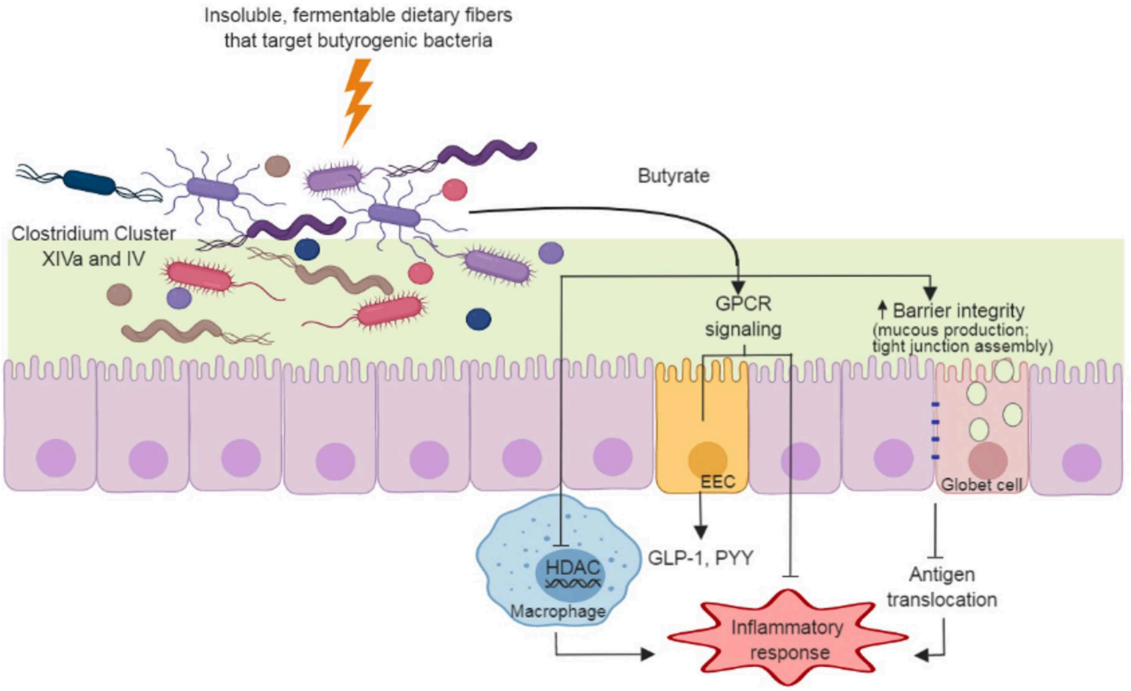

FIGURE 1 | Dietary fiber approach to increase gut-produced butyrate and pathways that have potential benefits to Parkinson's Disease. Insoluble dietary fibers with specific chemical structures are fermented by butyrate producers in the gut (e.g., Clostridium Cluster XIVa and IV species). The butyrate produced during fermentation supports gut barrier function through the stimulation of mucus production and tight junction assemblies, and minimizing antigen translocation and inflammation. Butyrate also regulates inflammatory pathways through G protein-coupled receptors (GPCR) in enterocytes and through inhibition of histone deacetylase (HDAC) in macrophages. GPCR signaling in enteroendocrine cells (EEC) induce secretion of hormones (e.g., glucagon-like peptide-1 [GLP-1] and peptide YY[PYY]) that act in many organs, including the brain.

increased colonic GLP-1 levels as well as upregulated GLP-1 receptors (GLP-1R) in the brain and resulted in improved neurobehavioral impairment (52).

Butyrate also influences histone acetylation, a post-translational modification that influences the propensity of a gene to be transcribed or repressed (53). Butyrate acts as a histone deacetylase inhibitor (HDACi) (54), attenuating production and secretion of pro-inflammatory cytokines in response to lipopolysaccharide stimuli in macrophages, complementing analogous modulation of inflammatory process via GPCRs (55) (**Figure 1**). Butyrate-targeted histone deacetylase inhibition is also neuroprotective against dopamine cell death (44) and DNA damage (42) *in-vitro*. In a rotenone-induced drosophila model of PD, sodium butyrate was able to improve locomotor deficits and reduce early mortality (40). Similar results were observed in a 6-hydroxydopamine-induced rat model of PD, in which sodium butyrate attenuated motor impairment and increased dopamine levels (45). In addition, Zhou et al. (43) showed that in a cell culture and a murine model of PD, sodium butyrate was able to up-regulate gene expression of DJ-1, a protein known to protect dopamine neurons from oxidative stress and moderate protein aggregation.

All animal studies using PD models utilized oral administration of sodium butyrate, rather than an approach using butyrogenic prebiotics. It should be noted that sodium butyrate is delivered differently to the body compared to microbiota-produced butyrate from prebiotic fermentation. Sodium butyrate is absorbed mostly in upper segments of the gastrointestinal tract, it leads to significant increases in plasma concentrations of butyrate (56). While this could result in direct actions in the brain, upper gastrointestinal tract absorption prevents most of the butyrate supplemented to reach the large intestine, where it has functions that could be relevant in PD (e.g., gut barrier function, regulation of inflammatory pathways, enteroendocrine hormone release). Microbiota-derived butyrate, on the other hand, generally is considered to act locally in the gut, with the remaining portion absorbed by the liver with no significant amounts reaching bloodstream (57). Interestingly, some reports show increased blood levels of circulating butyrate in healthy subjects in response to dietary fiber interventions (58–60), indicating that a portion of butyrate may escape liver absorption and could have a direct action in the brain. Inflammatory conditions may also cause an increase of SCFAs in peripheral venous blood (61), and therefore, the extent of microbiota produced-butyrate that reaches bloodstream in PD patients is still a matter of investigation. Overall, the use of prebiotic dietary fibers to increase butyrate in the colon could promote both localized and systemic effects (**Figure 1**), which seems like a promising approach in the management of PD. However, preclinical

studies are needed to evaluate how gut-derived butyrate affects PD pathophysiology.

Some controversy regarding the commonly accepted concept of anti-inflammatory and neuroprotective action of SCFAs was brought to light in a study using a mouse model of PD (62, 63). Sampson et al. (62) reported that the oral administration of a SCFA mixture, as well as a fecal transplant, to animals raised in a germ-free environment or antibiotic-treated, enhanced PD pathophysiology. It was not clear, however, if the SCFA mixture dosage utilized corresponds to levels that can be reached through gut-microbiota production. In this regard, oral administration of 100 mg/kg of sodium butyrate (NaB), but not 1,200 mg/kg, attenuated social deficits in an autism mouse model (64), indicating that distinct outcomes may take place by changing SCFA concentration. Another consideration is that orally delivered butyrate is mainly absorbed in the upper gastrointestinal tract and could have distinctly different outcomes from the colonic-produced butyrate.

BUTYROGENIC BACTERIA IN THE LARGE INTESTINE

A number of commensal gram-positive bacteria in the human gut possess the ability to produce butyrate. The majority of the butyrate producing bacteria belong to Clostridium Clusters IV and XIVa of the Firmicutes phylum. These clusters comprise highly oxygen-sensitive bacteria, which are estimated to significantly contribute to colonic butyrate production (65, 66). They also correspond to a numerically important portion of colonic bacteria. *Faecalibacterium prausnitzii* from Clostridium Cluster IV and *Eubacterium rectale* from Clostridium Cluster XIVa comprise up to 14 and 13%, respectively, of total fecal gut microbiota (67). Other major butyrogenic bacteria isolated from the human colon are *Roseburia* spp., *Eubacterium* spp., *Anaerostipes caccae*, *Butyrivibrio fibrisolvens*, *Coprococcus* spp. from Clostridium Cluster XIVa and *Subdoligranulum variabile* and *Anaerotruncus colihominis* from Clostridium Cluster IV (66). Many of commensal clostridial species preferentially colonize the mucus layer (e.g., *E. rectale, F. prausnitzii*, and *R. intestinalis*) which is in close proximity to gut epithelium. This strategic position favors butyrate interaction and uptake by intestinal cells, stimulating physiologic, metabolic and immunologic processes of health significance. Nonetheless, species such as *A. caccae* mostly inhabit the lumen of the colon where butyrate production helps to reduce luminal pH, preventing the growth pathogenic bacteria (68–70). Non-butyrogenic species also indirectly contribute to butyrate formation through production of other SCFA as a more acidic gut milieu favors the growth of butyrogenic species (71–73). Also, many butyrogenic bacteria utilize lactate and acetate from other bacteria to produce butyrate (66). The importance of such crossfeeding mechanisms to improve butyrate formation in the gut is still a matter of discussion as many butyrogenic bacteria occupy spatially distinct niches different than non-butyrogenic ones within the gut (70, 74, 75).

Depletion of butyrogenic bacteria from Clostridium Cluster IV and XIVa, especially those found nearly associated to the mucus layer is a common and potentially negative finding in the elderly (68). On top of that, PD patients show lower abundance of Lachnospiraceae family members (Clostridium Cluster XIVa) (6–8) and *Faecalibacterium* (Clostridium Cluster IV) (6, 8–10), as well as low production of all three SCFAs, including butyrate (9) compared to individuals of similar age.

PREBIOTIC DIETARY FIBER TARGETING BUTYROGENIC BACTERIA AND BUTYRATE PRODUCTION

Colonic bacteria produce butyrate mainly through dietary fiber fermentation, with proteolytic pathways contributing very little to overall butyrate production (65). Consumption of a meat-based diet for five consecutive days resulted in lower butyrate levels in fecal samples of healthy volunteers when compared to a plant-based diet. Butyrate reduction was accompanied by decrease in abundance of butyrogenic bacteria from Firmicutes, such as *Roseburia* and *E. rectale* (Clostridium Cluster XIVa). Another study with obese individuals showed that 4 weeks of a very low total carbohydrate intake (24 g/day), including low dietary fiber, resulted in a 4-fold decrease in *Roseburia*

TABLE 1 | Examples of insoluble substrates capable of promoting butyrogenic colonic bacteria.

Dietary fiber	Study design	Butyrogenic bacteria positively affected	Study
Chitin-glucan complexes	Fecal analysis from diet-induced obese mice	Clostridium Cluster XIVa, including *Roseburia* spp.	Neyrinck et al. (80)
β-1,3/1,6-D-glucan	*In vitro* human fecal fermentation	*Anaerostipes* spp. and *Roseburia*	Cantu-Jungles et al. (81)
Whole grain barley	Fecal analysis from healthy human subjects	*Eubacterium rectale*, *Roseburia faecis* and *Roseburia intestinalis*	Martinez et al. (82)
Wheat bran	Fecal analysis from obese males	Members from Lachnospiraceae family	Salonen et al. (83)
Acetylated galactoglucomannan and highly acetylated arabinoglucuronoxylan (AGX)	*In vitro* human fecal fermentation	*Faecalibacterium prausnitzii*	La Rosa et al. (84)
Wheat bran	*In vitro* human fecal fermentation	Members from Lachnospiraceae family and uncultured butyrate producers	Duncan et al. (85)
Coarse wheat bran	In *vitro* human fecal fermentation	*Coprococcus eutactus*, *Roseburia* and other Lachnospiraceae family members	Tuncil et al. (86)

spp. and *E. rectale* accompanied by the same magnitude reduction in butyrate fecal content (76). These data suggest that these colonic bacteria are particularly dependent upon dietary fiber consumption.

Contrary to what is found in Bacteroidetes (known as carbohydrate generalists, as many species have overlapping nutrient utilization abilities), available data suggest that, in addition to crossfeeding, butyrogenic bacteria are more specialized to degrade unique fiber structures. For example, Sheridan et al. (77) showed that even bacteria from the same *Roseburia* genus (Clostridium Cluster XIVa) present variable abilities to grow in distinct substrates in single cultures, with little overlapping in fiber utilization capabilities within species.

As previously discussed, fiber physical features are also related to its fermentation profile. Most bacteria attached to particles recovered from human feces belong to Firmicutes (mean 76.8% against only 18.5% Bacteroidetes), with high abundance of species from Clostridium Cluster IV and XIVa (74). *In vitro* fecal fermentation of wheat bran also showed that Clostridium Cluster XIVa dominated amongst particle-associated bacteria (78). As primary colonizers of insoluble substrates, these bacteria would hold a competitive advantage to degrade insoluble fermentable substrates. In fact, in pure cultures of *R. intestinalis* and *Bacteroides xylanisolvens*, the former was shown to be strongly associated with insoluble xylan, while *B. xylanisolvens* was enriched in solubilized xylan fractions (79).

Corroborating these results, many insoluble substrates such as chitin-glucan and β-glucan, as well as some cereals rich in insoluble fractions, were shown to increase butyrate and/or colonic butyrogenic bacteria (**Table 1**). Chitin-glucan complexes were shown to specifically increase Clostridium Cluster XIVa, including *Roseburia* spp. in high-fat (HF) diet-induced obese mice and promoted desirable metabolic outcomes (80). In our research group, insoluble β-glucans from fungi specifically increased *Anaerostipes* spp. (Clostridium Cluster XIVa) from <0.5% of the total bacteria in the initial inoculum to approximately 24% after fermentation of such fiber *in vitro* (81). This was accompanied by butyrate increase from 12.5 to 24–26% after β-glucan fermentation (81). Whole grain barley (82) and wheat bran (83, 85) were shown to be fermented by members of Lachnospiraceae family (Clostridium Cluster XIVa) in human colonic microbiota. Lignocellulosic dietary fibers from feedstocks such as galactoglucomannan and arabinoglucuronoxylan were shown to increase *Faecalibacterium prausnitzii* (Clostridium Cluster IV) (84). In an indirect way, acetate producers, such as *Ruminococcus bromii* through utilization of resistant starch, can promote butyrate production through cross-feeding (87). These studies confirm that insoluble polymers with distinct chemical structures boost divergent butyrogenic bacteria in the colon. Finally, besides solubility degree and chemical structure, particle size may be an important fiber characteristic to consider in butyrogenic prebiotic fiber design and selection. Tuncil et al. (86) showed that *in vitro* fecal fermentation of larger wheat bran particle size fractions led to higher butyrate production, as well as increases in some members of the Lachnospiraceae family (Clostridium Cluster XIVa). In contrast, smaller particles were associated with higher propionate production.

Overall, the few studies using dietary fiber treatment in PD patients have focused on intestinal constipation (39, 88) and its pharmacokinetic effects on drug absorption (88). Metabolites produced in the gut, and composition of gut microbiota in response to dietary fiber treatment, have not been assessed. Cross-sectional studies indicate that the microbial composition in PD patients present distinct composition from healthy controls (6–10). Although differences in microbial composition varies between PD and healthy controls across studies, all researchers report decreased abundance of butyrate producers, such as bacteria from Clostridium Cluster XIVa and/or IV (6–10). As butyrate is known to play important physiological roles both within the gastrointestinal tract and in diverse body sites, a dietary fiber approach targeting increases in colonic butyrogenic bacteria (**Figure 1**) could be beneficial to PD. Studies designed to evaluate dietary fiber effects on bacterial shifts and beneficial metabolite production, especially butyrate, as well as its relation to inflammation, gut permeability, and neurological outcomes in PD, should be conducted. Dietary fibers with specific chemical structures can be selected and/or designed to evaluate if a targeted colonic increase in butyrate and butyrate producers is beneficial to the management of PD outcomes beyond intestinal constipation.

CONCLUSION

Promoting increases in gut-derived butyrate is a promising approach in PD that could have implications in the management of gut and systemic disturbances. Prebiotic fiber features such as solubility degree, and chemical and physical structures may be important in allowing butyrogenic bacteria to compete against Gram-negative carbohydrate-utilizing bacteria for a more targeted prebiotic approach. The use of specific butyrogenic prebiotic fiber structures in PD models would allow for future pre-clinical studies to understand the effect of gut-produced butyrate in PD.

AUTHOR CONTRIBUTIONS

TC-J and HR wrote the manuscript. HR and BH revised the manuscript.

REFERENCES

1. Thomas B, Beal M. Parkinson's disease. *Hum Mol Genet.* (2007) 16:R138–94. doi: 10.1093/hmg/ddm159
2. Braak H, Del Tredici K. Nervous system pathology in sporadic Parkinson disease. *Neurology.* (2008) 70:1916–25. doi: 10.1212/01.wnl.0000312279.49272.9f
3. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* (2007) 33:599–614. doi: 10.1111/j.1365-2990.2007.00874.x
4. Lebouvier T, Chaumette T, Paillusson S, Duyckaerts C, Bruley des Varannes S, Neunlist M, et al. The second brain and Parkinson's disease. *Eur J Neurosci.* (2009) 30:735–41. doi: 10.1111/j.1460-9568.2009.06873.x

5. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE.* (2011) 6:e28032. doi: 10.1371/journal.pone.0028032
6. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord.* (2015) 30:1351–60. doi: 10.1002/mds.26307
7. Hill-Burns EM, Debelius JW, Morton JT, Wissemann WT, Lewis MR, Wallen ZD, et al. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Mov Disord.* (2017) 32:739–49. doi: 10.1002/mds.26942
8. Petrov VA, Saltykova IV, Zhukova IA, Alifirova VM, Zhukova NG, Dorofeeva YB, et al. Analysis of Gut Microbiota in Patients with Parkinson's Disease. *Bull Exp Biol Med.* (2017) 162:734–7. doi: 10.1007/s10517-017-3700-7
9. Unger MM, Spiegel J, Dillmann K-U, Grundmann D, Philippeit H, Bürmann J, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinson Relat Disord.* (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
10. Li W, Wu X, Hu X, Wang T, Liang S, Duan Y, et al. Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. *Sci China Life Sci.* (2017) 60:1223–33. doi: 10.1007/s11427-016-9001-4
11. Perez-Pardo P, Dodiya HB, Engen PA, Naqib A, Forsyth CB, Green SJ, et al. Gut bacterial composition in a mouse model of Parkinson's disease. *Benef Microbes.* (2018) 9:799–814. doi: 10.3920/BM2017.0202
12. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr.* (1995) 125:1401–12. doi: 10.1093/jn/125.6.1401
13. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* (2017) 14:491. doi: 10.1038/nrgastro.2017.75
14. Ciorba MA. A gastroenterologist's guide to probiotics. *Clin Gastroenterol Hepatol.* (2012) 10:960–8. doi: 10.1016/j.cgh.2012.03.024
15. Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, et al. Prebiotic effects: metabolic and health benefits. *Br J Nutr.* (2010) 104:S1–63. doi: 10.1017/S0007114510003363
16. Gibson GR, Fuller R. Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. *J Nutr.* (2000) 130:391S−5S. doi: 10.1093/jn/130.2.391S
17. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin Gastroenterol.* (2015) 31:69. doi: 10.1097/MOG.0000000000000139
18. Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front Microbiol.* (2016) 7:979. doi: 10.3389/fmicb.2016.00979
19. Zhang Y-J, Li S, Gan R-Y, Zhou T, Xu D-P, Li H-B. Impacts of gut bacteria on human health and diseases. *Int J Mol Sci.* (2015) 16:7493. doi: 10.3390/IJMS16047493
20. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* (2014) 426:3838–50. doi: 10.1016/j.jmb.2014.07.028
21. Codex Alimentarius Commission. *Report of the 30th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (ALINORM 09/32/26)*. Rome: Codex Alimentarius Commission (2009).
22. de Jesus Raposo MF, de Morais AMMB, de Morais RMSC. Emergent sources of prebiotics: seaweeds and microalgae. *Mar Drugs.* (2016) 14:27. doi: 10.3390/md14020027
23. Ruthes AC, Smiderle FR, Iacomini M. Mushroom heteropolysaccharides: a review on their sources, structure and biological effects. *Carbohydr Polym.* (2016) 136:358–75. doi: 10.1016/j.carbpol.2015.08.061
24. Grondin JM, Tamura K, Déjean G, Abbott DW, Brumer H. Polysaccharide utilization loci: fuelling microbial communities. *J Bacteriol.* (2018) 199:e00860-16. doi: 10.1128/JB.00860-16
25. Rose DJ, Patterson JA, Hamaker BR. Structural differences among alkali-soluble arabinoxylans from maize (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) brans influence human fecal fermentation profiles. *J Agric Food Chem.* (2010) 58:493–9. doi: 10.1021/jf9020416
26. Xu H. *Influence of the Structural Complexity of Cereal Arabinoxylans on Human Fecal Fermentation and Their Degradation Mechanism by Gut Bacteria*. Theses Diss Available from ProQuest (2012) Available online at: https://docs.lib.purdue.edu/dissertations/AAI3544591 (accessed January 28, 2019).
27. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res.* (2013) 54:2325–40. doi: 10.1194/jlr.R036012
28. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes.* (2017) 8:172–84. doi: 10.1080/19490976.2017.1290756
29. Zoetendal EG, de Vos WM. Effect of diet on the intestinal microbiota and its activity. *Curr Opin Gastroenterol.* (2014) 30:189–95. doi: 10.1097/MOG.0000000000000048
30. Vinolo MAR, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. *Nutrients.* (2011) 3:858–76. doi: 10.3390/nu3100858
31. Tedelind S, Westberg F, Kjerrulf M, Vidal A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. *World J Gastroenterol.* (2007) 13:2826–32. doi: 10.3748/wjg.v13.i20.2826
32. Liu T, Li J, Liu Y, Xiao N, Suo H, Xie K, et al. Short-chain fatty acids suppress lipopolysaccharide-induced production of nitric oxide and proinflammatory cytokines through inhibition of NF-κB pathway in RAW264.7 cells. *Inflammation.* (2012) 35:1676–84. doi: 10.1007/s10753-012-9484-z
33. Al-Asmakh M, Hedin L. Microbiota and the control of blood-tissue barriers. *Tissue Barriers.* (2015) 3:1039691. doi: 10.1080/21688370.2015.1039691
34. Hoyles L, Snelling T, Umlai U-K, Nicholson JK, Carding SR, Glen RC, et al. Microbiome-host systems interactions: protective effects of propionate upon the blood-brain barrier. *Microbiome.* (2018) 6:55. doi: 10.1186/s40168-018-0439-y
35. Oleskin AV, Shenderov BA. Neuromodulatory effects and targets of the SCFAs and gasotransmitters produced by the human symbiotic microbiota. *Microb Ecol Heal Dis.* (2016) 27:30971. doi: 10.3402/mehd.v27.30971
36. Strandwitz P. Neurotransmitter modulation by the gut microbiota. *Brain Res.* (2018) 1693:128–33. doi: 10.1016/j.brainres.2018.03.015
37. Grider JR, Piland BE. The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. *Am J Physiol Liver Physiol.* (2007) 292:G429–37. doi: 10.1152/ajpgi.00376.2006
38. Winge K, Rasmussen D, Werdelin LM. Constipation in neurological diseases. *J Neurol Neurosurg Psychiatry.* (2003) 74:13–9. doi: 10.1136/JNNP.74.1.13
39. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, Pusani C, et al. Probiotics and prebiotic fiber for constipation associated with Parkinson disease. *Neurology.* (2016) 87:1274–80. doi: 10.1212/WNL.0000000000003127
40. St. Laurent R, O'Brien LM, Ahmad ST. Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease. *Neuroscience.* (2013) 246:382–90. doi: 10.1016/j.neuroscience.2013.04.037
41. Mony L, Kew JN, Gunthorpe MJ, Paoletti P. Allosteric modulators of NR2B-containing NMDA receptors: molecular mechanisms and therapeutic potential. *Br J Pharmacol.* (2009) 157:1301–17. doi: 10.1111/j.1476-5381.2009.00304.x
42. Paiva I, Pinho R, Pavlou MA, Hennion M, Wales P, Schütz A-L, et al. Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage. *Hum Mol Genet.* (2017) 26:2231–46. doi: 10.1093/hmg/ddx114
43. Zhou W, Bercury K, Cummiskey J, Luong N, Lebin J, Freed CR. Phenylbutyrate up-regulates the DJ-1 protein and protects neurons in cell culture and in animal models of Parkinson disease. *J Biol Chem.* (2011) 286:14941–51. doi: 10.1074/jbc.M110.211029
44. Kidd SK, Schneider JS. Protection of dopaminergic cells from MPP+-mediated toxicity by histone deacetylase inhibition. *Brain Res.* (2010) 1354:172–8. doi: 10.1016/j.brainres.2010.07.041
45. Salama AF, Ibrahim W, Tousson E, Sakr S, Masoud A, Akela MA, et al. Epigenetic study of Parkinson's disease in experimental animal model. *Int J Clin Exp Neurol.* (2015) 3:11–20. doi: 10.12691/IJCEN-3-1-3
46. Roediger WEW. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology.* (1982) 83:424–9. doi: 10.1016/S0016-5085(82)80339-9

47. Husted AS, Trauelsen M, Rudenko O, Hjorth SA, Schwartz TW. GPCR-mediated signaling of metabolites. *Cell Metab.* (2017) 25:777–96. doi: 10.1016/J.CMET.2017.03.008
48. Kim MH, Kang SG, Park JH, Yanagisawa M, Kim CH. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology.* (2013) 145:396–406.e10. doi: 10.1053/j.gastro.2013.04.056
49. D'Souza WN, Douangpanya J, Mu S, Jaeckel P, Zhang M, Maxwell JR, et al. Differing roles for short chain fatty acids and GPR43 agonism in the regulation of intestinal barrier function and immune responses. *PLoS ONE.* (2017) 12:e0180190. doi: 10.1371/journal.pone.0180190
50. Christiansen CB, Gabe MBN, Svendsen B, Dragsted LO, Rosenkilde MM, Holst JJ. The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am J Physiol Liver Physiol.* (2018) 315:G53–65. doi: 10.1152/ajpgi.00346.2017
51. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes.* (2012) 61:364–71. doi: 10.2337/db11-1019
52. Liu J, Wang F, Liu S, Du J, Hu X, Xiong J, et al. Sodium butyrate exerts protective effect against Parkinson's disease in mice via stimulation of glucagon like peptide-1. *J Neurol Sci.* (2017) 381:176–81. doi: 10.1016/j.jns.2017.08.3235
53. Santos AL, Lindner AB. Protein posttranslational modifications: roles in aging and age-related disease. *Oxid Med Cell Longev.* (2017) 2017:1–19. doi: 10.1155/2017/5716409
54. Davie JR. Inhibition of histone deacetylase activity by butyrate. *J Nutr.* (2003) 133:2485S—93S. doi: 10.1093/jn/133.7.2485S
55. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci USA.* (2014) 111:2247–52. doi: 10.1073/pnas.1322269111
56. Egorin MJ, Yuan Z-M, Sentz DL, Plaisance K, Eiseman JL. Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats. *Cancer Chemother Pharmacol.* (1999) 43:445–53. doi: 10.1007/s002800050922
57. Knudsen KEB, Serena A, Canibe N, Juntunen KS. New insight into butyrate metabolism. *Proc Nutr Soc.* (2003) 62:81–6. doi: 10.1079/PNS2002212
58. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr.* (2005) 82:559–67. doi: 10.1093/ajcn.82.3.559
59. Priebe MG, Wang H, Weening D, Schepers M, Preston T, Vonk RJ. Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation. *Am J Clin Nutr.* (2010) 91:90–7. doi: 10.3945/ajcn.2009.28521
60. Wolever TM, Chiasson JL. Acarbose raises serum butyrate in human subjects with impaired glucose tolerance. *Br J Nutr.* (2000) 84:57–61. doi: 10.1017/S0007114500001239
61. Ktsoyan ZA, Mkrtchyan MS, Zakharyan MK, Mnatsakanyan AA, Arakelova KA, Gevorgyan ZU, et al. Systemic concentrations of short chain fatty acids are elevated in salmonellosis and exacerbation of familial mediterranean fever. *Front Microbiol.* (2016) 7:776. doi: 10.3389/fmicb.2016.00776
62. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell.* (2016) 167:1469–80.e12. doi: 10.1016/j.cell.2016.11.018
63. Mulak A. A controversy on the role of short-chain fatty acids in the pathogenesis of Parkinson's disease. *Mov Disord.* (2018) 33:398–401. doi: 10.1002/mds.27304
64. Kratsman N, Getselter D, Elliott E. Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model. *Neuropharmacology.* (2016) 102:136–45. doi: 10.1016/j.neuropharm.2015.11.003
65. Vital M, Karch A, Pieper DH. Colonic butyrate-producing communities in humans: an overview using omics data. *mSystems.* (2017) 2:e00130-17. doi: 10.1128/MSYSTEMS.00130-17
66. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett.* (2009) 294:1–8. doi: 10.1111/j.1574-6968.2009.01514.x
67. Walker AW, Duncan SH, Louis P, Flint HJ. Phylogeny, culturing, and metagenomics of the human gut microbiota. *Trends Microbiol.* (2014) 22:267–74. doi: 10.1016/J.TIM.2014.03.001
68. El Aidy S, Van den Abbeele P, Van de Wiele T, Louis P, Kleerebezem M. Intestinal colonization: how key microbial players become established in this dynamic process. *BioEssays.* (2013) 35:913–23. doi: 10.1002/bies.201300073
69. Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, et al. Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an *in vitro* gut model. *ISME J.* (2013) 7:949–61. doi: 10.1038/ismej.2012.158
70. Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathog.* (2013) 5:23. doi: 10.1186/1757-4749-5-23
71. Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol.* (2005) 71:3692–700. doi: 10.1128/AEM.71.7.3692-3700.2005
72. Belenguer A, Duncan SH, Holtrop G, Anderson SE, Lobley GE, Flint HJ. Impact of pH on lactate formation and utilization by human fecal microbial communities. *Appl Environ Microbiol.* (2007) 73:6526–33. doi: 10.1128/AEM.00508-07
73. Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol.* (2009) 11:2112–22. doi: 10.1111/j.1462-2920.2009.01931.x
74. Walker AW, Duncan SH, Harmsen HJM, Holtrop G, Welling GW, Flint HJ. The species composition of the human intestinal microbiota differs between particle-associated and liquid phase communities. *Environ Microbiol.* (2008) 10:3275–83. doi: 10.1111/j.1462-2920.2008.01717.x
75. Pereira FC, Berry D. Microbial nutrient niches in the gut. *Environ Microbiol.* (2017) 19:1366–78. doi: 10.1111/1462-2920.13659
76. Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol.* (2007) 73:1073–8. doi: 10.1128/AEM.02340-06
77. Sheridan PO, Martin JC, Lawley TD, Browne HP, Harris HMB, Bernalier-Donadille A, et al. Polysaccharide utilization loci and nutritional specialization in a dominant group of butyrate-producing human colonic Firmicutes. *Microb Genomics.* (2016) 2:e000043. doi: 10.1099/mgen.0.000043
78. Leitch ECM, Walker AW, Duncan SH, Holtrop G, Flint HJ. Selective colonization of insoluble substrates by human faecal bacteria. *Environ Microbiol.* (2007) 9:667–79. doi: 10.1111/j.1462-2920.2006.01186.x
79. Mirande C, Kadlecikova E, Matulova M, Capek P, Bernalier-Donadille A, Forano E, et al. Dietary fibre degradation and fermentation by two xylanolytic bacteria *Bacteroides xylanisolvens* XB1A ^{T} and *Roseburia intestinalis* XB6B4 from the human intestine. *J Appl Microbiol.* (2010) 109:451–60. doi: 10.1111/j.1365-2672.2010.04671.x
80. Neyrinck AM, Possemiers S, Verstraete W, De Backer F, Cani PD, Delzenne NM. Dietary modulation of clostridial cluster XIVa gut bacteria (*Roseburia* spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. *J Nutr Biochem.* (2012) 23:51–9. doi: 10.1016/j.jnutbio.2010.10.008
81. Cantu-Jungles TM, Ruthes AC, El-Hindawy M, Moreno RB, Zhang X, Cordeiro LMC, et al. *In vitro* fermentation of *Cookeina speciosa glucans* stimulates the growth of the butyrogenic *Clostridium cluster* XIVa in a targeted way. *Carbohydr Polym.* (2018) 183:219–29. doi: 10.1016/j.carbpol.2017.12.020
82. Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, et al. Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J.* (2013) 7:269–80. doi: 10.1038/ismej.2012.104

83. Salonen A, Lahti L, Salojärvi J, Holtrop G, Korpela K, Duncan SH, et al. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J.* (2014) 8:2218–30. doi: 10.1038/ismej.2014.63
84. La Rosa SL, Kachrimanidou V, Buffetto F, Pope PB, Pudlo NA, Martens EC, et al. Wood-Derived Dietary Fibers Promote Beneficial Human Gut Microbiota. *mSphere.* (2019) 4:18. doi: 10.1128/mSphere.00554-18
85. Duncan SH, Russell WR, Quartieri A, Rossi M, Parkhill J, Walker AW, et al. Wheat bran promotes enrichment within the human colonic microbiota of butyrate-producing bacteria that release ferulic acid. *Environ Microbiol.* (2016) 18:2214–25. doi: 10.1111/1462-2920.13158
86. Tuncil YE, Thakkar RD, Marcia ADR, Hamaker BR, Lindemann SR. Divergent short-chain fatty acid production and succession of colonic microbiota arise in fermentation of variously-sized wheat bran fractions. *Sci Rep.* (2018) 8:16655. doi: 10.1038/s41598-018-34912-8
87. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *mBio.* (2019) 10:e02566-18. doi: 10.1128/MBIO.02566-18
88. Astarloa R, Mena MA, Sánchez V, de la Vega L, de Yébenes JG. Clinical and pharmacokinetic effects of a diet rich in insoluble fiber on Parkinson disease. *Clin Neuropharmacol.* (1992) 15:375–80.

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Contributions of Gut Bacteria and Diet to Drug Pharmacokinetics in the Treatment of Parkinson's Disease

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Parkinson's disease is the second-most common neurodegenerative disorder worldwide. Besides deciphering the mechanisms that underlie the etiology of the disease, it is important to elucidate the factors that influence the efficacy of the treatment therapeutics. Levodopa, which remains the golden treatment of the disease, is absorbed in the proximal small intestine. A reduction in levodopa absorption, leads to reduction in striatal dopamine levels and, in turn, an "off"-episode. In fact, motor fluctuations represent a major problem during the progression of the disease and alteration between "on" (mobility often with dyskinesia) and "off" (immobility, akinesia) episodes contribute to a decreased quality of life. Dietary amino acids can interfere with the absorption of levodopa from the gut lumen and its transport through the blood brain barrier. In addition, higher abundance of specific gut bacteria that restrict levodopa absorption plays a significant role in motor fluctuations in a subset of Parkinson's disease patients. Here, we review the impact of factors potentially interfering with levodopa absorption, focusing on levodopa transport, diet, and gut bacterial interference with the bioavailability of levodopa.

Keywords: levodopa, transporters, bioavailability, small intestinal bacterial overgrowth, gut motility

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INTRODUCTION

Parkinson's disease (PD) is the second-most common neurodegenerative disorder worldwide (1). In 2015–2016, 6.1–6.2 million individuals were diagnosed with PD all over the globe (1, 2). The prevalence of PD globally increases with age and peaks at 1.5% between 85 and 89 years of age (2). During the progression of PD, patients encounter increasing severity of symptoms, which is associated with rising costs for medical treatment, hospitalizations and nursing home care (3), besides a significant decrease in the quality of life (3–6). The aggregation of α-synuclein in Lewy bodies and loss of dopaminergic neurons (pars compacta) in the substantia nigra is the main feature observed in PD patients (7). Although the exact factors contributing to the etiology of PD are not well understood, the gut microbiota is likely to be a key contributor. This is evident from the alteration in gut microbiota composition detected in fecal samples of PD patients compared to healthy controls (HC) (8–12). Moreover, the production of short-chain fatty acids (SCFAs), the main metabolic products produced by the large intestinal bacteria, is reduced in PD patients (12). The latter has been shown to be involved in α-synuclein pathology in the gut in mouse models (13) supporting the hypothesis that α-synuclein pathology starts in the enteric nervous system (14), which synergizes with the finding of α-synuclein aggregates in colon tissue and appendix prior to the onset of PD (15, 16). Equally important to elucidating the mechanisms involved in the cause

of PD is to uncover the microbial and dietary interference with the pharmacological treatment of the disease. Previous studies have shown that *Helicobacter pylori* (HP) can interfere with levodopa treatment and can bind to levodopa (3,4-dihydroxyphenylalanine; L-DOPA) (17, 18). Recently, we showed that bacteria can alter the levels of levodopa treatment in the gut (19) resulting in quenching the availability of the drug to be effective in the brain. This bacterial mediated reduction in levodopa absorbed from the small intestine would lead to reduction in striatal dopamine levels and an "off"-episode, especially in patients with advanced stage PD, who have a reduced capacity to store dopamine in the brain (20, 21). Besides, fluctuating levodopa plasma levels could result in increased pulsatile stimulation which is associated with dyskinesia (22). The pharmacological treatment of PD and the gastrointestinal (GI) dysfunction in PD have been extensively reviewed (23, 24), mainly from a clinical perspective. This review focuses on the impact of levodopa transport, gut bacterial degradation of PD medication, and its impact on drug bioavailability. Furthermore, we discuss the potential mediators that could lead to a vicious circle where certain conditions (i.e., proton pump inhibitors and gut motility) would favor the colonization of small-intestinal bacteria, ultimately restricting the absorption of levodopa.

ADMINISTRATION ROUTES AND TRANSPORT PROCESS OF LEVODOPA

The most common route for levodopa administration is orally via immediate-release or extended-release formulations of levodopa, where the latter might have potential benefits over other levodopa formulations, reviewed in Mittur et al. (25). Parenteral administration via subcutaneous injections are impossible due to the low solubility of levodopa (26) and continuous intravenous administration, although effective (27), is impractical, as it requires large volumes of daily injections. A promising alternative option to conventional levodopa therapy for advanced PD patients with motor fluctuations and dyskinesia is intestinal infusion of a levodopa/carbidopa gel via a nasoduodenal tube (28) or via gastrojejunostomy (22).

When levodopa is administered orally, it is absorbed in the proximal small intestine (29), where it has to be actively transported from the lumen over the intestinal epithelial barrier into the blood stream (30). To prevent peripheral and intestinal levodopa metabolism by DOPA decarboxylase (DDC), peripheral DDC inhibitors, such as carbidopa, are co-administered with levodopa. Levodopa (**Figure 1**) is a non-proteinogenic large neutral amino acid (LNAA), and is therefore transported by amino acid transporters in the GI-tract and at the blood brain barrier (BBB) (**Figure 2**). The human body contains at least 11 different epithelial amino acid transport systems expressed in the intestine, 10 of which are also expressed in the renal epithelia, which was thoroughly reviewed before (31). Only two amino acid transporters are expressed on the blood brain barrier (BBB), LAT1 (SLC7A5) and SNAT5/11 (SLC38A5/11) (32). The amino acid transporters, which are most likely responsible for the transport of levodopa from the GI-tract to the blood and over the BBB, based on *in vitro/ex vivo* studies, are discussed below and summarized in **Figure 2**.

As a model for the BBB, a mouse brain endothelial cell line (MBEC4), was tested for the expression of 4F2hc/LAT1 (SLC3A2/SLC7A5) and [^{3}H]-levodopa transport was evaluated in the presence of other amino acids (1:100 levodopa/amino acids). The study showed that tryptophan, tyrosine, phenylalanine, isoleucine, leucine, histidine, and 2-amino-2-norbornane-carboxylic acid (BCH), which is used as the defining synthetic amino acid for the L-system (consisting of LAT1 to 4) (33), inhibited at least 80% of the [^{3}H]-levodopa uptake independent of Na^+ (34). However, the potential contribution of 4F2hc/LAT2 (SLC3A2/SLC7A8) or other transporters were not addressed. Similar results were obtained in Caco2 cells (35–38), renal proximal tubular epithelial cells (39), and opossum kidney cells with either a high (HC) or a low (LC) Na^+ influx. Comparing the HC and LC cell lines indicated that there was a minor contribution of Na^+ dependent transport. The authors concluded that 4F2hc/LAT2 (apparent from BCH transport) and rBAT/$b^{0,+}$ (SLC3A1/SLC7A9; apparent from the uptake of the rBAT defining amino acid dimer, cystine) were involved in levodopa transport (40). Although these studies indicate which transporters are involved in levodopa transport in the GI-tract, renal epithelia and the BBB, it remains unclear which specific transporter is involved.

Studies using *Xenopus laevis* oocytes, an ideal single-cell expression system for transporters due to its relatively large size and low background activity (41), showed that 4F2hc/LAT1 (from rat C6 glioma cells) (42), 4Fhc/LAT2 (43), rBAT/$b^{0,+}$ (from rabbit intestine and human) (43, 44), and TAT1 (SLC16A10) (from rat intestine) (45) are independently responsible for levodopa transport. Only substrates with both positive and negative charges at the α-carbon (the relative positive and negative charges are from the amine-group and carboxyl-group from levodopa, respectively, **Figure 1**) are being able to be transported via 4F2hc/LAT1 (42). Importantly levodopa analogs (*m*-O-methylDOPA, α-methylphenylalanine, α-methyltyrosine, α-methylDOPA), gabapentin [γ-aminobutyric acid (GABA) analog], melphalan (a chemotherapeutic agent), and thyroid hormones (T3, triiodothyronine and T4, thyroxine) were able to inhibit transport of L-[^{14}C]-phenylalanine, and thus levodopa (42), showing the broad range of potential levodopa transport inhibitors. In fact, anti-thyroid treatment in a 70-year-old male subject with PD on levodopa treatment had a beneficial effect on the exaggerated Parkinsonian tremor (46). The authors could not explain why the Parkinsonian tremor was aggravated by the presence of hyperthyroidism. However, a plausible explanation, which was not discussed, is the interference of exaggerated thyroid hormone levels with levodopa uptake in the brain. Thus, hyperthyroidism, which is prevalent at higher age, should be considered in PD patients (46).

In *X. laevis* oocytes expressing TAT1, around 80% of L-[^{14}C]-tryptophan uptake was inhibited by tyrosine and tryptophan and about 40% was inhibited by phenylalanine, levodopa, and *m*-O-methylDOPA, indicating that TAT1 is an aromatic amino acid transporter partly responsible for levodopa uptake. Using N-acetylated amino acids, the authors concluded that

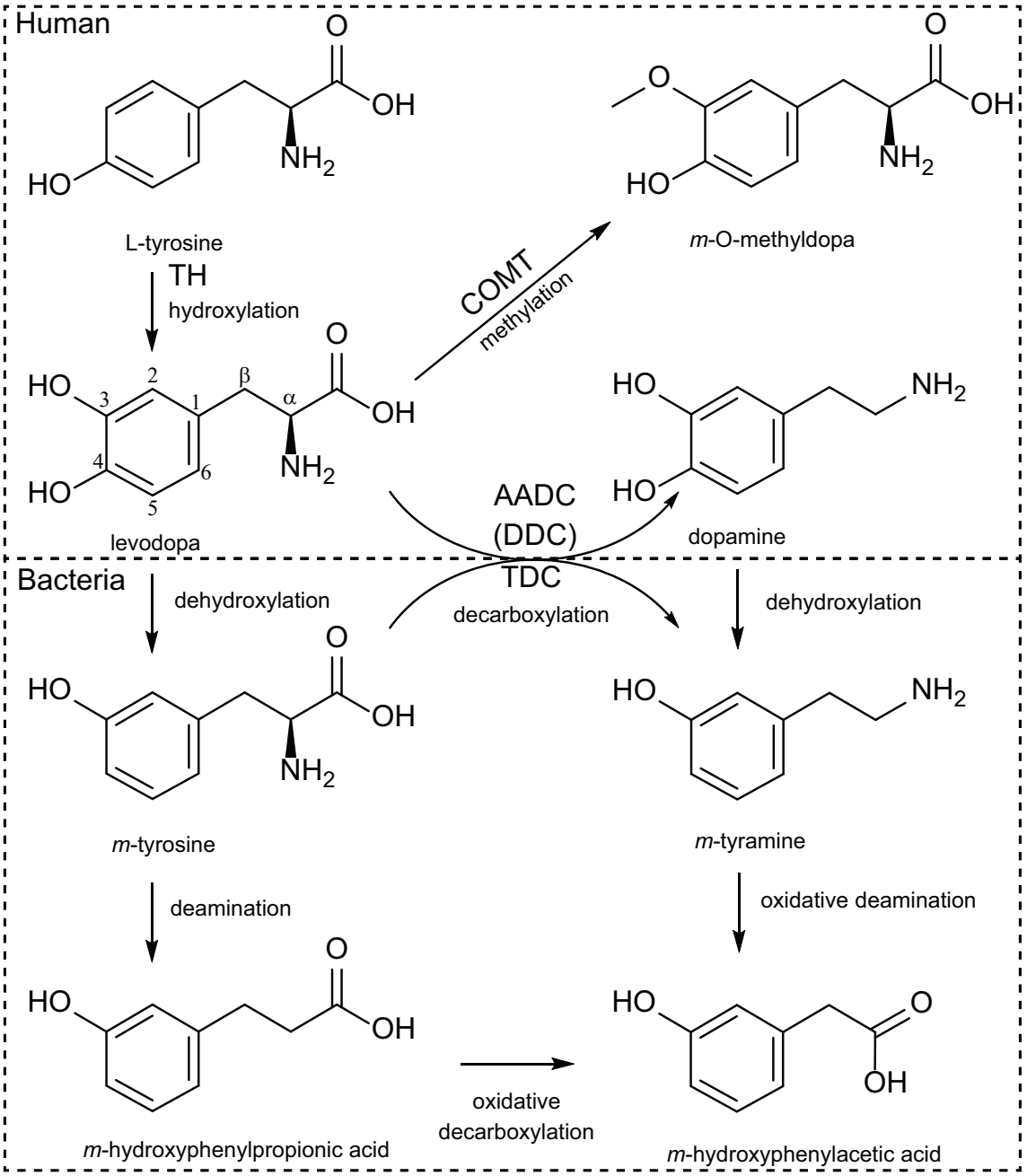

FIGURE 1 | Human and bacterial levodopa metabolism. Levodopa is produced by hydroxylation of the meta-position of the phenyl-ring from tyrosine by TH (tyrosine hydroxylase) using molecular oxygen. Sequentially levodopa can be decarboxylated to the active neurotransmitter dopamine by the AADC [aromatic amino acid decarboxylase, also known as DDC (DOPA decarboxylase)], or can be methylated by COMT (catechol-O-methyltransferase). Bacterial TDC (tyrosine decarboxylase) can decarboxylate (*m*-)tyrosine to (*m*-)tyramine but also levodopa to dopamine. Furthermore, bacteria can dehydroxylate the para-hydroxyl group of either levodopa or dopamine and can sequentially deaminate the dehydroxylated products.

the α-carboxyl group (**Figure 1**) is essential for substrate recognition by TAT1. Furthermore, it was shown that TAT1 is mainly expressed throughout in the rat GI-tract and in the liver, in particular, on the basolateral side of rat small intestine (45) (**Figure 2**). Using trans-well culturing and everted murine jejunal sacs, the authors concluded that 4F2hc/LAT2 (LAT1 was not tested) and TAT1 are responsible for the basolateral transport of levodopa (30). In contrast to 4F2hc/LAT1, 4F2hc/LAT2, and TAT1, which are expressed basolaterally, rBAT/$b^{0,+}$AT is expressed apically and thus is mainly responsible for levodopa absorption from the intestinal lumen. Further characterization of rBAT/$b^{0,+}$AT showed that the common co-administered

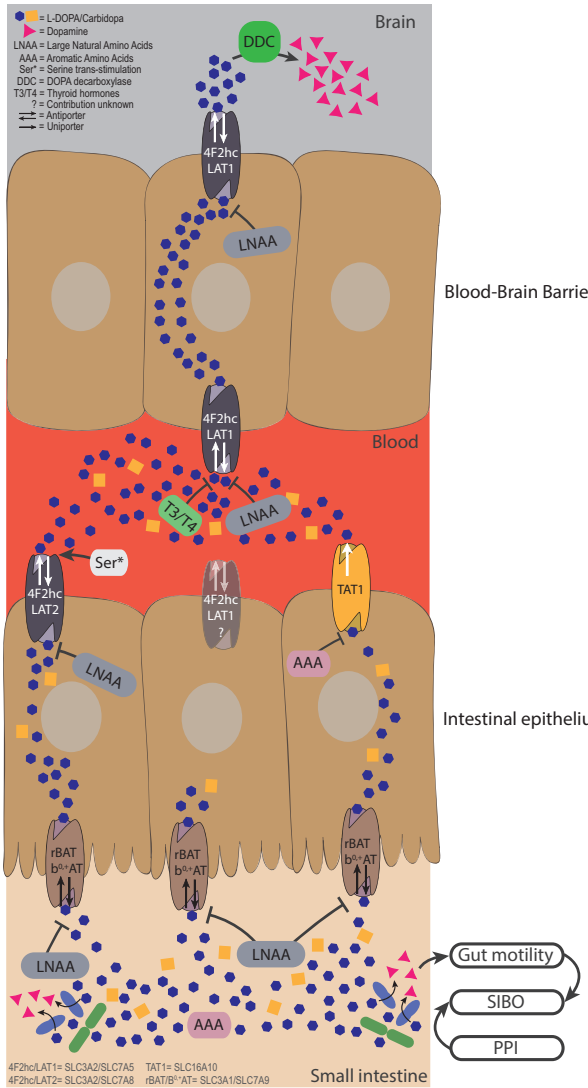

FIGURE 2 | Bacterial degradation and dietary components restrict levodopa transport. Levodopa is taken up in the small intestine by the apical transporter rBAT/$b^{0,+}$AT, and is sequentially is transported over the basolateral membrane by 4F2hc/LAT2 and TAT1. The uptake from the lumen can be compromised by LNAAs apically and by LNAAs and AAAs basolaterally. Bacterial degradation can interfere with levodopa before it is transported and elevate levels of dopamine in the lumen. Higher levels of luminal dopamine could affect the gut motility, which, in turn, could result in a state of small intestinal bacterial overgrowth, creating a vicious circle. The fraction of levodopa that ends up in the blood has to be transported over the BBB via 4F2hc/LAT1, which can be compromised by high levels of thyroid hormones (T3/T4), or LNAA. Serine left over from a late proteic meal, can trans-stimulate 4F2hc/LAT2 inducing higher efflux of levodopa in the circulation. Finally, the remaining levodopa will be converted to dopamine in the brain by DDC, to compensate the loss of striatal dopamine levels in PD patients.

inhibiters of peripheral levodopa degradation, carbidopa, benserazide (decarboxylase inhibitors) and entacapone [catechol-O-methyltransferase (COMT) inhibitor] were unable to compete with rBAT/$b^{0,+}$AT mediated levodopa transport, indicating that other transporters/mechanisms are involved in the uptake of peripheral levodopa metabolism inhibitors (30). The transport of levodopa via other apical transporters, PAT1, SIT1/ACE2, ASCT2, and B^0AT1/ACE2 (the main other natural amino acid transporter), expressed in *X. laevis* oocytes was investigated and showed that none of them was able to transport levodopa, indicating that rBAT/$b^{0,+}$AT is the main apical levodopa transporter (30) (**Figure 2**).

EFFECT OF DIET AND AGE ON THE BIOAVAILABILITY OF LEVODOPA

Early studies *in vivo*, using radiolabeled levodopa ([^{14}C]-levodopa) showed that ∼90% of the total radioactivity is transported into the circulatory system as measured in urine samples after 48 h (47–49). Notably, only ∼13% of the total radioactivity in blood plasma after the first hour was from intact levodopa, and decreased further overtime. When carbidopa was used in combination with levodopa the intact levodopa after the first hour increased to ∼43% (47). These studies indicate that less than half of the administered levodopa would reach the brain and that approximately 10% of the total levodopa radioactivity is not absorbed and could end up in fecal samples. Moreover, levels of unabsorbed levodopa increase over age. For example, a 10-fold increase (24.6–35.4% vs. 2.7–3.5% recovered radioactivity) in levels of levodopa (including its metabolites) were detected in fecal samples of old rats (0.5–2 years old) when compared with their younger counterparts (5–15 weeks old) after oral administration of [^{14}C]-levodopa (50). This was not related to an increased fecal excretion or decreased jejunal blood flow, suggesting that there is impaired uptake at older age (50). When levels of levodopa were measured over time in plasma (AUC), older animals (1–2 years) had a higher AUC and a longer half-life ($T_{1/2}$) of systemic levodopa compared to younger animals (9–26 weeks), suggesting an age-dependent slower total body clearance of levodopa (50). Furthermore the study showed that the intestinal metabolism (mainly by DDC), which prevents levodopa to reach the brain and decreases over age, contributes the most to the increased systemic availability of levodopa at older age (50). The decreased clearance of levodopa at higher age in rats is in agreement with a study performed in healthy human subjects, who were administered levodopa without DDC inhibitors (51). Coherently, a higher AUC and systemic levodopa bioavailability ($AUC_{oral}/AUC_{intravenous}$) for levodopa was observed in elderly (71.0 years $n = 9$) compared to young subjects (21.8 years $n = 8$). Administration of carbidopa diminished the differences in systemic levodopa bioavailability between the two groups, while a higher AUC was still observed in the elderly group. This suggests a lower systemic clearance at higher age because carbidopa abolished the age differences in systemic levodopa bioavailability (51). In PD patients, age correlated significantly with higher levodopa (supplied with DDC inhibitor) AUC and decrease in clearance (52, 53). However, the high scatter in the correlation ($r^2 = 0.15$–0.24) from that study implies that other factors besides age contribute to the variation among PD patients in the pharmacokinetics of levodopa (52).

Indeed, impaired uptake of [^{14}C]-levodopa into the brain was observed when rats were supplied intravenously with the amino acids, phenylalanine, tryptophan, and to a lesser extent histidine (54). The same effects were reported in humans, for example, a clinical study showed that PD patients ($n = 9$), who received levodopa/carbidopa intravenously directly after a protein rich meal (containing LNAAs) or administration of LNAAs, had increased Parkinsonian symptoms. Similarly, when levodopa/carbidopa was taken orally, levodopa absorption from the intestine was delayed after a protein-rich meal (55). When levodopa/benzerazide (another DDC inhibitor) was infused intraduodenally, motor functions decreased after protein ingestion (56), indicating fluctuation in levodopa uptake in the brain. Nonetheless no decrease in levodopa absorption was observed (56) suggesting that the variability in plasma LNAAs, absorbed from the intestine, could be responsible for the fluctuating levodopa uptake in the brain (57). The authors concluded that during ingestion of regular (hospital) diets, 10% of the levodopa brain uptake variability is explained by LNAAs in plasma and the other 90% by levodopa plasma levels (57). These hospital diets contained 2–3.7-fold less LNAAs compared to other human studies [615 ± 105 μM (57) compared to 1,235–1,973 μM (55), 1,615–2,012 μM (58), 1,624–2,292 μM (56)] indicating that high LNAA levels do interfere with levodopa absorption in PD patients but are not solely responsible for the "on"–"off" fluctuations observed in PD patients. Notably, cationic (lysine) or small (glycine) amino acids had no effect on the "on"–"off" fluctuations (55). Using regional jejunal perfusion of levodopa in healthy human subjects it was shown that the LNAA L-leucine interfered with the levodopa absorption from small intestine (59), at least at high concentrations. This finding supports the involvement of the L-transport system for levodopa transport (as described above) from the intestine to the blood circulation, and, ultimately, to the brain (**Figure 2**).

In vitro data and clinical investigations on the effect of amino acids on the transport and bioavailability of levodopa clearly indicate that amino acids can interfere with the uptake of levodopa from the lumen or the systemic circulation. Therefore, low protein diets (LPD) or protein redistribution diets (PDR), where all dietary protein is ingested only during the evening meal, are proposed for PD patients with motor fluctuations (60). Refined physiologically based pharmacokinetic (PBPK) modeling for GI absorption (WB-ACAT, Whole Body—Advanced Compartmental Absorption and Transit Model) combined with dynamic flux balance analysis (which measures the flow of metabolites through a metabolic network) on an epithelial cell (sIEC) model for small intestine segmented into 7 parts (WB-ACAT-sIEC), was used to investigate the spatiotemporal relationship between amino acids and levodopa uptake kinetics (61). Simulation of levodopa absorption during an aproteic or proteic meal showed that that dietary intervention would be beneficial for PD patients with Hoehn and Yahr scale 3/4 (HY3/4; HY describes the disease progression from (mild = 1) to severe = 5) (61). These findings are in agreement with the guidelines for PD treatment, where dietary interventions are proposed for advanced PD patients (20, 21). Comparing a LPD (*in silico* administration of 0.8 g/kg amino acids together with 200 mg levodopa) vs. a PRD (assuming a high fraction of amino acids present in the systemic circulation before the morning levodopa dose) in the WB-ACAT-sIEC model showed a cumulative increase in AUC of levodopa during PRD. Furthermore, the AUC after a morning levodopa dose was higher (11.23%) during PRD than during a fasting state, which was attributed to a higher influx of residual systemic LNAA from the last protein meal taken the evening before levodopa administration. This higher influx through the basolateral antiporter induced a higher efflux of levodopa (trans-stimulation) into the circulation (61) (**Figure 2**). Although PRD could provide short-term benefits as evident by the reported response rates of >80% (60), it might not provide a long-term solution as it is undesired by patients and is an imbalanced diet (20, 21) that results in weight loss among patients (60). Extending the WB-ACAT-sIEC model with kidney and brain compartments and setting the objective function (a desired outcome) for optimizing levodopa transport across the BBB revealed that threonine, serine and asparagine resulted in the highest brain bioavailability of levodopa. This led the authors to propose that a serine-rich meal taken after the last levodopa treatment could be beneficial for the levodopa bioavailability (61). Nonetheless, sensitivity analyses (i.e., the variable that contributes most to the dependent outcome) showed that intestinal loss of levodopa was the most influential factor on levodopa bioavailability (61). Indeed, changes in the levels of levodopa in the small intestine are affected by gut bacterial interference (17, 19), as discussed in the next section.

GUT BACTERIAL INTERFERENCE WITH LEVODOPA BIOAVAILABILITY

Levodopa is a non-proteinogenic amino acid produced by the hydroxylation at the meta-position of the phenyl ring of tyrosine. Subsequently, levodopa can be converted to dopamine by DDC or to *m*-O-methylDOPA by COMT methylating of the *m*-hydroxyl group in the human body (**Figure 1**). The microbiota also poses enzymes able to perform similar or additional reactions, which metabolize levodopa. In the early 70s, a study, comparing the metabolic profile of germ-free and conventional rats, showed production of *m*-hydroxyphenylacetic acid and *m*-hydroxyphenylpropionic acid (**Figure 1**) only in conventional rats when fed with levodopa, suggesting that a bacterial dehydroxylation reaction was involved (62). When rat caecal content was incubated with levodopa or dopamine for 6 days also *m*-tyramine was found, confirming earlier findings in humans (63). Metabolites were detected over periods of 3 days in the urine indicating that the detected metabolites could originate from in the large intestine, which is supported by the caecal incubations (62). Since the main site of levodopa absorption is the proximal small intestine, it is unlikely that bacterial metabolism of levodopa in the large intestine would affect the drug bioavailability. Therefore, it is crucial to investigate potential bacterial interference with levodopa treatment in the proximal small intestine.

Recently, we showed that gut bacteria harboring tyrosine decarboxylases (TDC), mainly enterococci, can effectively decarboxylate levodopa to dopamine in the small intestine of rat. The study concluded that the natural variation of the *tdc*-gene negatively correlated with the levodopa levels in the blood of rats and positively correlated with the daily dose requirement of levodopa in PD patients (19). High abundance of these bacteria in PD patients, which could be caused by small intestinal overgrowth (SIBO), could have implications on the absorption of levodopa from the small intestine (**Figure 2**). To assess the contribution of those bacteria to the bioavailability of levodopa in PD patients, we are currently performing further clinical studies.

In healthy conditions, SIBO is prevented by the ileocecal valve, pancreatic enzyme activity, gut motility and gastric acid (64). Importantly in PD patients, the prevalence of gut motility dysfunction (constipation) and proton pump inhibitor (PPI) usage is relatively high (77.1 and 39.6% respectively, $n = 39$) (65) and is associated with SIBO (66). In patients ($n = 200$) with gastroesophageal reflux disease using PPIs, varying from 2 months to 7 years, SIBO was detected in 50% of the cases and was significantly higher than in healthy controls ($n = 50$) (66). Studies looking at the alteration of the microbiota in subjects using PPIs showed increased levels of Bacilli (including *Lactobacillus*, *Staphylococcus,* and *Enterococcus*) in fecal samples (67, 68). In duodenal samples, SIBO was also observed in 56% of patients on PPIs ($n = 25$) and included mainly genera from the Bacilli class (69). Bacterial species from the Bacilli class are of importance as they harbor TDCs, which are able to interfere with levodopa levels (19). When SIBO is eradicated in PD patients with *Helicobacter pylori* infection using rifaximin, a common non-absorbable antibiotic used to treat SIBO (70), motor fluctuations were improved as apparent from the significant decreased delayed "on" episodes/day and daily "off" time, although no significant increase in levodopa pharmacokinetics was observed (71). The underlying explanation of improved motor fluctuations following SIBO eradication remains to be elucidated. However, a plausible explanation is that eradication of bacterial degradation of levodopa in the small intestine altered levels of the levodopa metabolite, dopamine, in the small intestinal lumen (19), and/or eliminated SIBO-induced small intestinal inflammation (71).

In 2001, investigators observed a clinical improvement in PD patients after treatment with antibiotics used to eradicate *Helicobacter pylori* in two almost identical reports. When HP-infections were treated, the mean AUC of levodopa in the blood significantly increased by ∼1.2-fold. A UPDRS-III motor examination showed indeed a significant decrease in motor score (72, 73). A follow-up study confirmed these findings in a larger cohort ($n = 17$) and showed that either 2 weeks or 3 months after HP eradication, PD patients had higher levodopa blood levels (AUC) and lower UPDRS-III motor scores compared to before the eradication (18). Other studies did not find a significant difference in pharmacokinetics (74) or LEDD (levodopa equivalent daily dose) (75, 76) of levodopa between PD patients tested positive or negative for HP infection. In addition, no motor improvement (UPDSR-III) was found after HP eradication in 34 patients (75). Despite the discrepancy among studies, *Helicobacter pylori* might still play a significant role in drug absorption. The mechanism of *Helicobacter pylori* affecting the levodopa absorption is unclear, one possible explanation for altered drug absorption might be the gastric acidity, which is altered by *Helicobacter pylori* infection and therefore interferes with drug pharmacokinetics of levodopa, delavirdine, and thyroxine (77). Interestingly, an *in vitro* study showed that adhesins exposed on the outer membrane of *Helicobacter pylori* might bind to levodopa and therefore might contribute to the lower pharmacokinetics in *Helicobacter pylori* infected PD patients (17). No follow-up studies were published and it remains to be elucidated which adhesin(s) are responsible for binding levodopa. Besides, whether the antibiotic cocktail used to treat *Helicobacter pylori* infections (1,000/500 mg amoxicillin/clarithromycin) could also eradicate other bacterial species in the small intestine, which might interfere with the availability of levodopa, and thus could be the actual reason behind the observed increase in blood levels of levodopa, was not investigated.

EFFECT OF DOPAMINE AND DOPAMINE AGONISTS ON GUT MOTILITY

Bacterial species from the Bacilli class, especially enterococci, are able to produce luminal dopamine (19). Importantly, dopamine and their agonists have been shown to affect the gut motility (discussed below), which could potentially favor the colonization of levodopa decarboxylating bacteria (19) (**Figure 2**). In addition, the dopamine agonists, which are usually used in combination with levodopa treatment, could have a similar effect on influencing gut motility to favor colonization of specific bacterial species. Therefore, studies investigating the effects of dopamine on gut motility of rodents, dogs, and humans were reviewed, with a complete overview in **Table 1**.

Using electrical field stimulation (EFS) on longitudinal muscle strips of guinea pig ileum in organ baths, dopamine (1–100 μM) and bromocriptine (0.15–15 μM), a dopamine agonist used in PD treatment, inhibited the cholinergic twitch up to ∼46 and ∼82%, respectively. Neither dopamine antagonists, metoclopramide nor pimozide prevented the observed inhibition by dopamine or bromocriptine. When using the α-adrenoceptor antagonist, phentolamine, only the observed inhibition of dopamine but not of bromocriptine was rescued, indicating that dopamine acts through the α-adrenoceptors (78). The same conclusions on the inhibitory effect of dopamine were shown in an almost identical study using ileum of guinea pig (79). Notably, tyramine, a product of bacterial TDC, resulted in similar inhibitions of cholinergic twitch (79). Dopamine, bromocriptine, and to a lesser extent tyramine, were also able to relax methacholine-contracted jejunal tissues from guinea pig (80). In rats, dopamine initiated directly a short longitudinal contraction followed by relaxation within 5 min in the duodenum and jejunum. However, in the ileum, only relaxations were observed (81). In addition, dopamine had also an inhibitory effect on the spontaneous contractions of longitudinal muscle strips from rat distal colon (82). The motility of mouse longitudinal fixed ileum (83), circular muscle strips of colon (84) and longitudinal fixed colon (85)

TABLE 1 | Studies investigating the effects of dopamine and dopamine agonists on gut motility in rodents, dogs and humans.

Study	Organism	Method	Tissue	Effect on motility	Tested agonists (μM)	Dopamine receptor antagonist (μM)	Adrenergic receptor antagonist (μM)	Other inhibitors	Effect inhibited by	Conclusion
Zar et al. (78)	Guinea pig	Organ bath	Ileum; longitudinal muscle; electrical field stimulation	Relaxation	Dopamine (1–100), Bromocriptine (0.15–15)	Pimozide (1)	Phentolamine (5), Metoclopramide (90)	None	Phentolamine (only DA)	Inhibition of longitudinal muscle motility through α-adrenergic receptors
Görich et al. (79)	Guinea pig	Organ bath	Ileum; longitudinal fixation (reserpine pretreatment)	Inhibitory	Dopamine, Noradrenaline, Clonidine (and tyramine) (1–100)	Metoclopramide (1–30), sulpiride (1–300), domperidone (0.01–1), pimozide (0.01–0.1) cis-flupentixol (0.1–1)	Tolazoline (0.3–3)	Reserpine (VMAT2 inhibitor)	Metoclopramide, sulpiride, tolazoline	Inhibition of motility by all compounds tested. Potentially through α-adrenergic receptors. The potency (pA2*) of metoclopramide and sulpiride was not different between dopamine or norepinephrine, indicating an α-adrenergic inhibition, confirmed by tolazoline
Lucchelli et al. (80)	Guinea pig	Organ bath	Jejunum; longitudinal fixation; methacholine induced contraction	Relaxation	Dopamine (1–000), Apomorphine (3–100), Bromocriptine (1–56), Fenoldopam (1,000), [and tyramine 1–3,000 (data not shown)]	Haloperidol (1,3), *cis*-flupenhixol (1), SCH-23390 (1,3)	Phentolamine (1,3), propranolol (0.3,1,3,10)	Reserpine (I.P. 5 mg/kg), TTX (0.3)	Phentolamine (only ∼7%) and propranolol (up to ∼45%)	Relaxation of tissue of all tested compounds (Reserpine, had no effect on DA induced relaxation, and a minor effect on the others). Slight inhibition of phentolamine (α-adrenoceptor antagonist) and propranolol (ß-adrenoceptor antagonist). Inconclusive which receptor is involved
Kirschstein et al. (81)	Rat	Organ bath	Duodenum, jejunum, ileum; longitudinal fixation	Relaxation and Constriction	Dopamine (100)	SCH-23390 (1), raclopride (1)	Propranolol (3), Prazosin (30)	None	All tested	Contraction and relaxation observed in duodenum and jejunum, relaxation only observed in Ileum. Contraction inhibition by SCH-23390 and raclopride, relaxation inhibition by propranolol and prazosin
Zhang et al. (82)	Rat	Organ bath	Distal colon; longitudinal strips	Inhibitory	Dopamine (3–30)	SCH-23390 (10), Supiride (10)	Not tested	TTX (1)	SCH-23390	Dopamine inhibited the spontaneous contractions with EC50=8.3μM and was not affected by TTX. The inhibitory affect was affected only by D1R antagonist SCH-23390
Zizzo et al. (83)	Mouse	Organ bath	Ileum; longitudinal fixation	Inhibitory	Dopamine (1–300), SKF-38393 (0.003–100)	SCH-23390 (3,10), Sulpiride (10), Domperidone (5)	Propranolol (10) SR-59230A (0.1), Phentolamine, (10) Yohimbine (10)	DDA (10), Apamin (0.1), Charybdotoxin (0.1), Iberiotoxin (0,1), TTX (1), L-NAME (100), Atropine(1), DPCPX (10), DMPX (10), MRS-1220 (0.1), Methysergide (1)	SR-59230, Phentolamine, Yohimbine (at high concentration of DA), SCH-23390 and SCH-23390 in combination with Sulpiride or Domperidone	Contractibility was inhibited by dopamine and SKF-38933 (D1R agonist), at high concentrations adrenoceptor antagonists (SR-59230, phentolamine, yohimbine) slightly prevented the inhibitory effect of dopamine. D2 antagonists sulpiride and domperidone had little effect on the inhibitory effect of dopamine, except when combined with SCH-23390 (D1R antagonist) which induced a stronger effect then SCH-23390 alone. Suggesting a synergic contribution of D1 and D2 receptors

(Continued)

TABLE 1 | Continued

Study	Organism	Method	Tissue	Effect on motility	Tested agonists (μM)	Dopamine receptor antagonist (μM)	Adrenergic receptor antagonist (μM)	Other inhibitors	Effect inhibited by	Conclusion
Auteri et al. (84)	Mouse	Organ bath	Colon; circular muscle strips; Carbachol precontracted or electrical field stimulation	Relaxation/ Inhibitory	Dopamine (1–300), SKF-38393 (up to 100), bromocriptine (0.3–100), isoproterenol	SCH-23390 (3), domperidone (5)	Prazosin (1), Yohimbine (1), propranolol (1), SR-59230A (0.1)	TTX (1), ω-conotoxin (0.1), SNX-482 (0.1), ω-agatoxin TK (0.1), L-NAME (100) MRS-2179 (1),	Domperidone (during carbochol contraction); SCH-23390 (during electrical field stimulation)	Relaxation induced by DA via a D2-like receptors; Not dependent on NO or P2Y1 receptors; Not affected by adrenergic antagonists; not dependent on enteric neuronal action potential or on modulation of neurotransmitter release; SCH-23390 increased basal tone and the amplitude of the spontaneous contractions; Relaxation of bromocriptine is inhibited by domperidone
Walker et al. (85)	Mouse	Organ bath	Distal colon (WT and DAT-/-); Longitudinal fixation; Electrical field stimulation	Inhibitory	Dopamine (0.01–300)	SCH-23390 (10), sulpiride (10)	Not tested	None	SCH-23390/sulpiride	Dopamine was only tested on WT distal colon and showed a inhibitory effect (EC50 = 4.5 μM), which was slightly abolished by SCH-23390/sulpiride mixture (EC50 = 12.9 μM, single applications of antagonist were not performed)
Fioramonti et al. (86)	Dog	Implanted Ni/Cr electrodes	Duodenum and jejunum	Inhibitory	Intracerebroventricularly dopamine (10 ug/kg); Intravenous dopamine (100 μg/kg)	None	None	None	NA	Decreased the duration of the migrating motor complex episodes in the small intestine 1 h before a meal compared to controls (from 9.4 to 3.4 h and 7.8 to 2.4 h in duodenum and jejunum), although intravenously (100 μg/kg) this effect was not observed
Bueno et al. (87)	Dog	Implanted strain gauge transducers	Ascending, traverse, descending colon	Inhibitory and Inducing	Iv injections of dopamine at 1 mg/kg/h or bromocriptine 40 ug/kg/h	Haloperidol (0.2 mg/kg)	Phentolamine (0.1 mg/kg), Tolazoline (2 mg/kg), Prazosin (0.2 mg/kg), propranolol (0.5 mg/kg)	None	Phentolamine, prazosin and haloperidol for dopamine inhibitory effect,	Dopamine had a inhibitory effect on the ascending and transverse colon and a inducing effect on the descending colon MMCs. Bromocriptine had a inducing effect in the whole colon MMCs; Potentially through adrenergic and dopaminergic action
Marzio et al. (88)	Human, healthy	Intestinal radiopaque tube consisting of four polyvinyl catheters with 4 side openings equally spread perfused with 1.59 ml/min with distilled water. Closure of the openings gives rise 100 mm hg/sec	Duodenum, proximal jejunum	Inducing	Intravenously dopamine 5 μg/kg/min for 15 min	Domperidon (10 mg) and sulpiride (100 mg)	None	None	Domperidon and sulpiride	Dopamine induced phase-III like MMCs in the duodenum, similar to spontaneous phase-III MMCs, although a slight longer period of complete inhibition after phase-III MMCs; Domperidon and sulpiride prevented the inducing phase-III MMCs effect

(Continued)

TABLE 1 | Continued

Study	Organism	Method	Tissue	Effect on motility	Tested agonists (μM)	Dopamine receptor antagonist (μM)	Adrenergic receptor antagonist (μM)	Other inhibitors	Effect inhibited by	Conclusion
Marzio et al. (89)	Human, healthy	Nasoduodenal probe consisting of 5 polyethylene catheters with evenly spaced openings 20 cm apart continuously perfused with 0.5 ml/min distilled water	Stomach, Duodenum, Proximal Jejunum	Inducing/Inhibitory	Intravenously dopamine 5 μg/kg/min for 15 min	Domperidon (20 mg)	None	None	Domperidon	Dopamine induced phase-III like MMCs during fed state in the small intestine, which was inhibited by domperidone, and decreased the motility of the stomach. After the phase-III MMCs a short period of complete quiescence was observed
Levein et al. (90)	Human, healthy	Paracetamol AUC; orocaecal transit time	Mouth -> Ileum	Inhibitory	Intravenously dopamine 5 μg/kg/min	None	None	None	NA	Dopamine reduced the AUC(60 min) of paracetamol significantly, associated with a delayed gastric emptying; OCT time was significantly longer then controls indicating a delayed gastric emptying and gut motility
Dive et al. (91)	Human, critically ill adults under mechanical ventilation without suffering from active gastro-intestinal disease	Multilumen tube consisting of polyvinyl catheters with side openings, 1.5 cm apart for stomach and 10 cm apart for duodenum continuously perfused with 0.2 ml/min distilled water	Stomach, duodenum	Inhibitory/Inducing	Intravenously dopamine 4 μg/kg/min	None	None	None	NA	Decreased number of contractions in the gastric antrum (only significant during fasting) and induced phase III motor activity in the duodenum (only significant during feeding)

pA2, the concentration that produces a 2-fold shift in the agonist concentration-response curve;* ***Dopaminergic antagonists:* *SCH-23390, D1 receptor antagonist; Domperidone, Haloperidol, Metoclopramide, Pimozide, Raclopride, Sulpiride, D2 receptor antagonist; cis-flupentixol, D1 and D2 receptor antagonist;* ***Adrenergic antagonists****: Tolazoline, Phentolamine, Prazosin, α1 adrenergic receptor antagonist; Yohimbine, α2 adrenergic receptor antagonist; Propranolol, ß adrenergic receptor antagonist; SR-59230A, β3-adrenoceptor antagonist;* ***Other antagonists and inhibitors****: Apamin, SK_{Ca} channel blocker; Atropine, Muscarinic receptor blocker; Carbachol, Cholinergic agonist; Charybdotoxin, IK_{Ca}-Bk_{Ca} channel blocker; DDA, Adenylyl cyclase inhibitor; DMPX, Adenosine A2 receptor antagonist; DPCPX, Adenosine A1 receptor antagonist; Iberiotoxin, BK_{Ca} channel blocker; L-NAME, NO synthase inhibitor; Methysergide, 5-HT receptor antagonist; MRS-1220, Adenosine A3 receptor antagonist; MRS-2179, Purinergic P2Y1 receptor antagonist; Reserpine, VMAT inhibitor; SNX-482, P/Q-type Ca^{2+} channel blocker; TTX, Na^{+}voltage-gated neural channel blocker; ω-agatoxin TK, R-type Ca^{2+} channel blocker; ω-conotoxin, N-type Ca^{2+} channel blocker.*

were all inhibited by dopamine and in the latter study also by bromocriptine, attributed to dopaminergic and/or adrenergic receptors. In dogs, the gut motility of the small intestine (86) and the colon (87) was monitored *in vivo* using implanted electrodes. Injection of dopamine (10 μg/kg) intracerebroventricularly 1 h before a meal decreased the duration of the migrating motor complex (MMC; intestinal motility pattern of the interdigestive state) episodes in the small intestine compared to controls, although this effect was not observed when dopamine was injected intravenously (100 μg/kg) (86). In the colon, a similar inhibition was observed, although with a 10 times higher concentration of dopamine (1 mg/kg/h) injected intravenously (87). Importantly, bromocriptine had an opposite effect, where it induced the colon motility instead (87). In fasted human subjects, intravenous administration of dopamine (75 μg/kg in 15 min) induced phase-III like MMCs (last phase in the MMC cycle which consists of strong contractions to completely occlude the lumen) in the duodenum (88), which is in contrast to the previous studies in rodents (organ bath experiments) and dogs. The MMCs were similar to spontaneous phase-III MMCs, although with a slight longer period of complete inhibition after phase-III MMCs (88). Similar results were found in terminally ill patients (91). A follow up study in humans during fed state showed that dopamine disrupted the fed state MMCs and induced phase-III like MMCs, followed by a short period of complete quiescence (phase-I like MMCs), which was inhibited by the dopamine receptor D2 blocker (DRD2) domperidone, suggesting the involvement of peripheral D2 receptors (89). Lastly, when the gut motility was investigated using orocaecal transit time (OCT) and paracetamol pharmacokinetics as gastric emptying marker during intravenous injection of dopamine (90), a reduction in the $AUC_{t=60min}$ of paracetamol was observed. This suggests that dopamine causes delayed OCT time, which could be due to delayed gastric emptying and a decrease in gut motility (90). Functional studies investigating the dopamine receptors in the GI-tract of mouse showed that the dopamine receptor D2 (Drd2) is important for gut motility. Mice lacking Drd2, but not Drd3, receptor showed an increased gut transit time compared to the controls (92) suggesting that endogenous dopamine has an inhibitory effect on intestinal motility (92). The findings confirm the earlier organ bath experiments with rodent tissue. In summary, these studies (**Table 1**) show that in rodents and dogs the GI motility is inhibited by dopamine through dopaminergic and adrenergic receptors.

In contrast, in humans, dopamine seems to inhibit stomach motility and induce phase-III like MMCs followed by a short time of quiescence through dopaminergic receptors. A potential explanation of the discrepancy among the human and the animal studies might be the experimental setup. In rodents, dissected intestinal parts were placed in an organ bath *ex vivo* and in dogs electrodes were implanted on the basal side of segments of the GI-tract (86, 87). In contrast, in human studies, nasojejunal luminal-tubes consisting of catheters with side openings were fluoroscopically placed in the GI-tract and perfused with 0.2–1.59 mL/min water (88, 89, 91). The latter might induce an altered gut motility *per se* in a non-physiological manner. More studies should be conducted to test the effects of dopamine on the gut motility in humans, and especially in PD patients, who might already have an altered gut motility (4).

CONCLUSIONS AND FUTURE PERSPECTIVES

The "on"/"off" motor fluctuations in PD patients are highly dependent on the pharmacological treatment and factors contributing to its efficacy. Dietary amino acids and gut bacterial interference with levodopa treatment can contribute to the reduction of levodopa dosage absorbed in the small intestine, thereby restrict the effectiveness of the treatment. Especially luminal dopamine, which is produced by gut bacterial degradation of levodopa and is affecting the gut motility, would enhance the overgrowth of these bacteria in the small intestine and result in a vicious circle that enhances SIBO. The effect of dopamine on (small) intestinal motility, urges the investigation of the effect luminal dopamine and dopamine agonists on the gut motility of PD patients. Finally, it is crucial to accurately measure levels of SIBO in PD patients, especially in those who administer PPIs, and to diagnose other possible underlying diseases, such as hyperthyroidism. These precautions will help reduce the factors contributing to compromised levodopa bioavailability and the unwarranted side effects that result from increased frequency of dosage treatment regimen.

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REFERENCES

1. Feigin VL, Abajobir AA, Abate KH, Abd-Allah F, Abdulle AM, Abera SF, et al. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Neurol*. (2017) 16:877–97. doi: 10.1016/S1474-4422(17)30299-5
2. Dorsey ER, Elbaz A, Nichols E, Abd-Allah F, Abdelalim A, Adsuar JC, et al. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. (2018) 17:939–53. doi: 10.1016/S1474-4422(18)30295-3
3. Kowal SL, Dall TM, Chakrabarti R, Storm MV, Jain A. The current and projected economic burden of Parkinson's disease in the United States. *Mov Disord*. (2013) 28:311–8. doi: 10.1002/mds.25292
4. Barone P, Antonini A, Colosimo C, Marconi R, Morgante L, Avarello TP, et al. The PRIAMO study: a multicenter assessment of nonmotor symptoms and their impact on quality of life in Parkinson's disease. *Mov Disord*. (2009) 24:1641–9. doi: 10.1002/mds.22643

5. Chapuis S, Ouchchane L, Metz O, Gerbaud L, Durif F. Impact of the motor complications of Parkinson's disease on the quality of life. *Mov Disord*. (2005) 20:224–30. doi: 10.1002/mds.20279
6. Martinez-Martin P, Rodriguez-Blazquez C, Kurtis MM, Chaudhuri KR. The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. *Mov Disord*. (2011) 26:399–406. doi: 10.1002/mds.23462
7. Braak H, Tredici K, Del Rüb U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
8. Hill-Burns EM, Debelius JW, Morton JT, Wissemann WT, Lewis MR, Wallen ZD, et al. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Mov Disord*. (2017) 32:739–49. doi: 10.1002/mds.26942
9. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord*. (2015) 30:1351–60. doi: 10.1002/mds.26307
10. Qian Y, Yang X, Xu S, Wu C, Song Y, Qin N, et al. Alteration of the fecal microbiota in Chinese patients with Parkinson's disease. *Brain Behav Immun*. (2018) 70:194–202. doi: 10.1016/j.bbi.2018.02.016
11. Scheperjans F, Aho V, Pereira PAB, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord*. (2015) 30:350–8. doi: 10.1002/mds.26069
12. Unger MM, Spiegel J, Dillmann K, Grundmann D, Philippeit H, Bürmann J, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat Disord*. (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
13. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*. (2016) 167:1469–80.e12. doi: 10.1016/j.cell.2016.11.018
14. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol*. (2007) 33:599–614. doi: 10.1111/j.1365-2990.2007.00874.x
15. Killinger BA, Madaj Z, Sikora JW, Rey N, Haas AJ, Vepa Y, et al. The vermiform appendix impacts the risk of developing Parkinson's disease. *Sci Transl Med*. (2018) 10:eaar5280. doi: 10.1126/scitranslmed.aar5280
16. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's Disease? Evidence from 3 cases. *Mov Disord*. (2012) 27:716–9. doi: 10.1002/mds.25020
17. Niehues M, Hensel A. *In-vitro* interaction of L-dopa with bacterial adhesins of Helicobacter pylori: an explanation for clinicial differences in bioavailability? *J Pharm Pharmacol*. (2009) 61:1303–7. doi: 10.1211/jpp/61.10.0005
18. Pierantozzi M, Pietroiusti A, Brusa L, Galati S, Stefani A, Lunardi G, et al. Helicobacter pylori eradication and l-dopa absorption in patients with PD and motor fluctuations. *Neurology*. (2006) 66:1824–9. doi: 10.1212/01.wnl.0000221672.01272.ba
19. van Kessel SP, Frye AK, El-Gendy AO, Castejon M, Keshavarzian A, van Dijk G, et al. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nat Commun*. (2019) 10:310. doi: 10.1038/s41467-019-08294-y
20. Olanow CW, Koller WC. An algorithm (decision tree) for the management of Parkinson's disease: treatment guidelines. *Am Acad Neurol Neurol*. (1998) 50:S1–57. doi: 10.1212/WNL.50.3_Suppl_3.S1
21. Olanow CW, Watts RL, Koller WC. An algorithm (decision tree) for the management of Parkinson's disease (2001): treatment guidelines. *Neurology*. (2001) 56:S1–88. doi: 10.1212/WNL.56.suppl_5.S1
22. Olanow CW, Kieburtz K, Odin P, Espay AJ, Standaert DG, Fernandez HH, et al. Continuous intrajejunal infusion of levodopa-carbidopa intestinal gel for patients with advanced Parkinson's disease: a randomised, controlled, double-blind, double-dummy study. *Lancet Neurol*. (2014) 13:141–9. doi: 10.1016/S1474-4422(13)70293-X
23. Connolly BS, Lang AE. Pharmacological treatment of Parkinson disease: a review. *JAMA*. (2014) 311:1670–83. doi: 10.1001/jama.2014.3654
24. Fasano A, Visanji NP, Liu LWC, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol*. (2015) 14:625–39. doi: 10.1016/S1474-4422(15)00007-1
25. Mittur A, Gupta S, Modi NB. Pharmacokinetics of Rytary®, an extended-release capsule formulation of carbidopa–levodopa. *Clin Pharmacokinet*. (2017) 56:999–1014. doi: 10.1007/s40262-017-0511-y
26. Lundqvist C. Continuous levodopa for advanced Parkinson's disease. *Neuropsychiatr Dis Treat*. (2007) 3:335–48.
27. Hardie RJ, Lees AJ, Stern GM. On-off fluctuations in Parkinson's disease. A clinical and neuropharmacological study. *Brain*. (1984) 107 (Pt 2):487–506. doi: 10.1093/brain/107.2.487
28. Nyholm D, Nilsson Remahl AIM, Dizdar N, Constantinescu R, Holmberg B, Jansson R, et al. Duodenal levodopa infusion monotherapy vs oral polypharmacy in advanced Parkinson disease. *Neurology*. (2005) 64:216–23. doi: 10.1212/01.WNL.0000149637.70961.4C
29. Gundert-Remy U, Hildebrandt R, Stiehl A, Weber E, Zürcher G, Da Prada M. Intestinal absorption of levodopa in man. *Eur J Clin Pharmacol*. (1983) 25:69–72. doi: 10.1007/BF00544017
30. Camargo SMR, Gotze O, Singer D, Verrey F, Vuille-dit-Bille RN, Ramadan T, et al. The molecular mechanism of intestinal levodopa absorption and its possible implications for the treatment of Parkinson's disease. *J Pharmacol Exp Ther*. (2014) 351:114–23. doi: 10.1124/jpet.114.216317
31. Bröer S. Amino acid transport across mammalian intestinal and renal epithelia. *Physiol Rev*. (2008) 88:249–86. doi: 10.1152/physrev.00018.2006
32. Geier EG, Chen EC, Webb A, Papp AC, Yee SW, Sadee W, et al. Profiling solute carrier transporters in the human blood-brain barrier. *Clin Pharmacol Ther*. (2013) 94:636–9. doi: 10.1038/clpt.2013.175
33. Wade LA, Katzman R. Synthetic amino acids and the nature of L-DOPA transport at the blood-brain barrier. *J Neurochem*. (1975) 25:837–42. doi: 10.1111/j.1471-4159.1975.tb04415.x
34. Kageyama T, Nakamura M, Matsuo A, Yamasaki Y, Takakura Y, Hashida M, et al. The 4F2hc/LAT1 complex transports L-DOPA across the blood-brain barrier. *Brain Res*. (2000) 879:115–21. doi: 10.1016/S0006-8993(00)02758-X
35. Fraga S, Serrão MP, Soares-da-Silva P. L-type amino acid transporters in two intestinal epithelial cell lines function as exchangers with neutral amino acids. *J Nutr*. (2002) 132:733–8. doi: 10.1093/jn/132.4.733
36. Fraga S, Sampaio-Maia B, Serrão MP, Soares-da-Silva P. Regulation of apical transporter of L-DOPA in human intestinal Caco-2 cells. *Acta Physiol Scand*. (2002) 175:103–11. doi: 10.1046/j.1365-201X.2002.00974.x
37. Fraga S, Serrão MP, Soares-da-Silva P. The L-3,4-dihydroxyphenylalanine transporter in human and rat epithelial intestinal cells is a type 2 hetero amino acid exchanger. *Eur J Pharmacol*. (2002) 441:127–35. doi: 10.1016/S0014-2999(02)01416-4
38. Fraga S, Pinho MJ, Soares-da-Silva P. Expression of LAT1 and LAT2 amino acid transporters in human and rat intestinal epithelial cells. *Amino Acids*. (2005) 29:229–33. doi: 10.1007/s00726-005-0221-x
39. Pinho MJ, Serrão MP, Gomes P, Hopfer U, Jose PA, Soares-Da-Silva P. Over-expression of renal LAT1 and LAT2 and enhanced L-DOPA uptake in SHR immortalized renal proximal tubular cells. *Kidney Int*. (2004) 66:216–26. doi: 10.1111/j.1523-1755.2004.00722.x
40. Gomes P, Soares-da-Silva P. Na+-independent transporters, LAT-2 and b0,+, exchange L-DOPA with neutral and basic amino acids in two clonal renal cell lines. *J Membr Biol*. (2002) 186:63–80. doi: 10.1007/s00232-001-0136-8
41. Bröer S. Xenopus laevis Oocytes. *Methods Mol Biol*. (2010) 637:295–310. doi: 10.1007/978-1-60761-700-6_16
42. Uchino H. Transport of amino acid-related compounds mediated by L-type Amino Acid Transporter 1 (LAT1): insights into the mechanisms of substrate recognition. *Mol Pharmacol*. (2002) 61:729–37. doi: 10.1124/mol.61.4.729
43. Quiñones H, Collazo R, Moe OW. The dopamine precursor l -dihydroxyphenylalanine is transported by the amino acid transporters rBAT and LAT2 in renal cortex. *Am J Physiol Physiol*. (2004) 287:F74–80. doi: 10.1152/ajprenal.00237.2003
44. Ishii H, Sasaki Y, Goshima Y, Kanai Y, Endou H, Ayusawa D, et al. Involvement of rBAT in Na+-dependent and -independent transport of the neurotransmitter candidate L-DOPA in Xenopus laevis oocytes injected with rabbit small intestinal epithelium poly A+ RNA. *Biochim Biophys Acta Biomembr*. (2000) 1466:61–70. doi: 10.1016/S0005-2736(00)00171-1
45. Kim DK, Kanai Y, Chairoungdua A, Matsuo H, Cha SH, Endou H. Expression cloning of a Na + -independent aromatic amino acid transporter with structural similarity to H + /Monocarboxylate transporters. *J Biol Chem*. (2001) 276:17221–8. doi: 10.1074/jbc.M009462200

46. Kim HT, Edwards MJ, Lakshmi Narsimhan R, Bhatia KP. Hyperthyroidism exaggerating parkinsonian tremor: a clinical lesson. *Parkinsonism Relat Disord*. (2005) 11:331–2. doi: 10.1016/j.parkreldis.2005.01.009
47. Bianchine JR, Messiha FS, Hsu TH. Peripheral aromatic L-amino acids decarboxylase inhibitor in parkinsonism. II. Effect on metabolism of L-2-^{14}C-dopa. *Clin Pharmacol Ther*. (1972) 13:584–94. doi: 10.1002/cpt1972134584
48. Morgan JP. Metabolism of levodopa in patients with Parkinson's disease. *Arch Neurol*. (1971) 25:39. doi: 10.1001/archneur.1971.00490010049007
49. Sasahara K, Nitanai T, Habara T, Kojima T, Kawahara Y, Morioka T, et al. Dosage form design for improvement of bioavailability of levodopa IV: possible causes of low bioavailability of oral levodopa in dogs. *J Pharm Sci*. (1981) 70:730–3. doi: 10.1002/jps.2600700705
50. Iwamoto K, Watanabe J, Yamada M, Atsumi F, Matsushita T. Effect of age on gastrointestinal and hepatic first-pass effects of levodopa in rats. *J Pharm Pharmacol*. (1987) 39:421–5. doi: 10.1111/j.2042-7158.1987.tb03413.x
51. Robertson D, Wood N, Everest H, Monks K, Waller D, Renwick A, et al. The effect of age on the pharmacokinetics of levodopa administered alone and in the presence of carbidopa. *Br J Clin Pharmacol*. (1989) 28:61–9. doi: 10.1111/j.1365-2125.1989.tb03506.x
52. Contin M, Riva R, Martinelli P, Albani F, Baruzzi A. Effect of age on the pharmacokinetics of oral levodopa in patients with Parkinson's disease. *Eur J Clin Pharmacol*. (1991) 41:463–6. doi: 10.1007/BF00626370
53. Nagayama H, Ueda M, Kumagai T, Tsukamoto K, Nishiyama Y, Nishimura S, et al. Influence of ageing on the pharmacokinetics of levodopa in elderly patients with Parkinson's disease. *Park Relat Disord*. (2011) 17:150–2. doi: 10.1016/j.parkreldis.2010.11.002
54. Daniel PM, Moorhouse RS, Pratt OE. Letter: do changes in blood levels of other aromatic aminoacids influence levodopa therapy? *Lancet*. (1976) 1:95. doi: 10.1016/S0140-6736(76)90194-X
55. Nutt JG, Woodward WR, Hammerstad JP, Carter JH, Anderson JL. The on–off phenomenon in Parkinson's disease. *N Engl J Med*. (1984) 310:483–8. doi: 10.1056/NEJM198402233100802
56. Frankel JP, Kempster PA, Bovingdon M, Webster R, Lees AJ, Stern GM. The effects of oral protein on the absorption of intraduodenal levodopa and motor performance. *J Neurol Neurosurg Psychiatry*. (1989) 52:1063–7. doi: 10.1136/jnnp.52.9.1063
57. Nutt JG, Woodward WR, Carter JH, Trotman TL. Influence of fluctuations of plasma large neutral amino acids with normal diets on the clinical response to levodopa. *J Neurol Neurosurg Psychiatry*. (1989) 52:481–7. doi: 10.1136/jnnp.52.4.481
58. Leenders KL, Poewe WH, Palmer AJ, Brenton DP, Frackowiak RS. Inhibition of L-[18F]fluorodopa uptake into human brain by amino acids demonstrated by positron emission tomography. *Ann Neurol*. (1986) 20:258–62. doi: 10.1002/ana.410200212
59. Lennernas H, Nilsson D, Aquilonius S, Ahrenstedt O, Knutson L, Paalzow L. The effect of L-leucine on the absorption of levodopa, studied by regional jejunal perfusion in man. *Br J Clin Pharmacol*. (1993) 35:243–50. doi: 10.1111/j.1365-2125.1993.tb05691.x
60. Cereda E, Barichella M, Pedrolli C, Pezzoli G. Low-protein and protein-redistribution diets for Parkinson's disease patients with motor fluctuations: a systematic review. *Mov Disord*. (2010) 25:2021–34. doi: 10.1002/mds.23226
61. Guebila BM, Thiele I. Model-based dietary optimization for late-stage, levodopa-treated, Parkinson's disease patients. *Npj Syst Biol Appl*. (2016) 2:16013. doi: 10.1038/npjsba.2016.13
62. Goldin BR, Peppercorn MA, Goldman P. Contributions of host and intestinal microflora in the metabolism of L-dopa by the rat. *J Pharmacol Exp Ther*. (1973) 186:160–6.
63. Sandler M, Goodwin BL, Ruthven CRJ, Calne DB. Therapeutic implications in Parkinsonism of m-tyramine formation from L-dopa in man. *Nature*. (1971) 229:414–6. doi: 10.1038/229414a0
64. Quigley EMM, Quera R. Small intestinal bacterial overgrowth: roles of antibiotics, prebiotics, and probiotics. *Gastroenterology*. (2006) 130:78–90. doi: 10.1053/j.gastro.2005.11.046
65. Gabrielli M, Bonazzi P, Scarpellini E, Bendia E, Lauritano EC, Fasano A, et al. Prevalence of small intestinal bacterial overgrowth in Parkinson's disease. *Mov Disord*. (2011) 26:889–92. doi: 10.1002/mds.23566
66. Lombardo L, Foti M, Ruggia O, Chiecchio A. Increased incidence of small intestinal bacterial overgrowth during proton pump inhibitor therapy. *Clin Gastroenterol Hepatol*. (2010) 8:504–8. doi: 10.1016/j.cgh.2009.12.022
67. Freedberg DE, Toussaint NC, Chen SP, Ratner AJ, Whittier S, Wang TC, et al. Proton pump inhibitors alter specific taxa in the human gastrointestinal microbiome: a crossover trial. *Gastroenterology*. (2015) 149:883–5.e9. doi: 10.1053/j.gastro.2015.06.043
68. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. *Gut*. (2016) 65:740–8. doi: 10.1136/gutjnl-2015-310376
69. Fried M, Siegrist H, Frei R, Froehlich F, Duroux P, Thorens J, et al. Duodenal bacterial overgrowth during treatment in outpatients with omeprazole. *Gut*. (1994) 35:23–6. doi: 10.1136/gut.35.1.23
70. Steffen R. Rifaximin: a nonabsorbed antimicrobial as a new tool for treatment of travelers' diarrhea. *J Travel Med*. (2001) 8:S34–9. doi: 10.1111/j.1708-8305.2001.tb00545.x
71. Fasano A, Bove F, Gabrielli M, Petracca M, Zocco MA, Ragazzoni E, et al. The role of small intestinal bacterial overgrowth in Parkinson's disease. *Mov Disord*. (2013) 28:1241–9. doi: 10.1002/mds.25522
72. Pierantozzi M, Pietroiusti A, Galante A, Sancesario G, Lunardi G, Fedele E, et al. Helicobacter pylori-induced reduction of acute levodopa absorption in Parkinson's disease patients. *Ann Neurol*. (2001) 50:686–7. doi: 10.1002/ana.1267
73. Pierantozzi M, Pietroiusti A, Sancesario G, Lunardi G, Fedele E, Giacomini P, et al. Reduced L-dopa absorption and increased clinical fluctuations in Helicobacter pylori-infected Parkinson's disease patients. *Neurol Sci*. (2001) 22:89–91. doi: 10.1007/s100720170061
74. Narozanska E, Białecka M, Adamiak-Giera U, Gawronska-Szklarz B, Sołtan W, Schinwelski M, et al. Pharmacokinetics of levodopa in patients with parkinson disease and motor fluctuations depending on the presence of helicobacter pylori infection. *Clin Neuropharmacol*. (2014) 37:96–9. doi: 10.1097/WNF.0000000000000037
75. Lee WY, Yoon WT, Shin HY, Jeon SH, Rhee PL. Helicobacter pylori infection and motor fluctuations in patients with Parkinson's disease. *Mov Disord*. (2008) 23:1696–700. doi: 10.1002/mds.22190
76. Rahne KE, Tagesson C, Nyholm D. Motor fluctuations and Helicobacter pylori in Parkinson's disease. *J Neurol*. (2013) 260:2974–80. doi: 10.1007/s00415-013-7089-6
77. Lahner E, Annibale B, Delle Fave G. Systematic review: heliocobacter pylori infection and impaired drug absorption. *Aliment Pharmacol Ther*. (2009) 29:379–86. doi: 10.1111/j.1365-2036.2008.03906.x
78. Zar MA, Ebong O, Bateman DN. Effect of metoclopramide in guinea-pig ileum longitudinal muscle: evidence against dopamine-mediation. *Gut*. (1982) 23:66–70. doi: 10.1136/gut.23.1.66
79. Görich R, Weihrauch TR, Kilbinger H. The inhibition by dopamine of cholinergic transmission in the isolated guinea-pig ileum. Mediation through alpha-adrenoceptors Naunyn Schmiedebergs. *Arch Pharmacol*. (1982) 318:308–12. doi: 10.1007/BF00501170
80. Lucchelli A, Boselli C, Grana E. Dopamine-induced relaxation of the guinea-pig isolated jejunum is not mediated through dopamine receptors. *Pharmacol Res*. (1990) 22:433–44. doi: 10.1016/1043-6618(90)90750-8
81. Kirschstein T, Dammann F, Klostermann J, Rehberg M, Tokay T, Schubert R, et al. Dopamine induces contraction in the proximal, but relaxation in the distal rat isolated small intestine. *Neurosci Lett*. (2009) 465:21–6. doi: 10.1016/j.neulet.2009.08.080
82. Zhang X, Guo H, Xu J, Li Y, Li L, Zhang X, et al. Dopamine receptor D1 mediates the inhibition of dopamine on the distal colonic motility. *Transl Res*. (2012) 159:407–14. doi: 10.1016/j.trsl.2012.01.002
83. Zizzo MG, Mulè F, Mastropaolo M, Serio R. D1 receptors play a major role in the dopamine modulation of mouse ileum contractility. *Pharmacol Res*. (2010) 61:371–8. doi: 10.1016/j.phrs.2010.01.015
84. Auteri M, Zizzo MG, Amato A, Serio R. Dopamine induces inhibitory effects on the circular muscle contractility of mouse distal colon via D1- and D2-like receptors. *J Physiol Biochem*. (2016) 73:395–404. doi: 10.1007/s13105-017-0566-0
85. Walker JK, Gainetdinov RR, Mangel AW, Caron MG, Shetzline MA. Mice lacking the dopamine transporter display altered regulation of distal

colonic motility. *Am J Physiol Gastrointest Liver Physiol*. (2000) 279:G311–8. doi: 10.1152/ajpgi.2000.279.2.G311
86. Fioramonti J, Fargeas MJ, Honde C, Bueno L. Effects of central and peripheral administration of dopamine on pattern of intestinal motility in dogs. *Dig Dis Sci*. (1984) 29:1023–7. doi: 10.1007/BF01311254
87. Bueno L, Fargeas MJ, Fioramonti J, Honde C. Effects of dopamine and bromocriptine on colonic motility in dog. *Br J Pharmacol*. (1984) 82:35–42. doi: 10.1111/j.1476-5381.1984.tb16439.x
88. Marzio L, Neri M, Di Giammarco AM, Cuccurullo F, Lanfranchi GA. Dopamine-induced migrating myoelectrical complex-like activity in human duodenum. *Dig Dis Sci*. (1986) 31:349–54. doi: 10.1007/BF01311668
89. Marzio L, Neri M, Pieramico O, Donne MD, Peeters TL, Cuccurullo F. Dopamine interrupts gastrointestinal fed motility pattern in humans. *Dig Dis Sci*. (1990) 35:327–32. doi: 10.1007/BF01537410
90. Levein NG, Thörn SE, Wattwil M. Dopamine delays gastric emptying and prolongs orocaecal transit time in volunteers. *Eur J Anaesthesiol*. (1999) 16:246–50. doi: 10.1097/00003643-199904000-00006
91. Dive A, Foret F, Jamart J, Bulpa P, Installé E. Effect of dopamine on gastrointestinal motility during critical illness. *Intensive Care Med*. (2000) 26:901–7. doi: 10.1007/s001340051279
92. Li ZS, Schmauss C, Cuenca A, Ratcliffe E, Gershon MD. Physiological modulation of intestinal motility by enteric dopaminergic neurons and the D2 receptor: analysis of dopamine receptor expression, location, development, and function in wild-type and knock-out mice. *J Neurosci*. (2006) 26:2798–807. doi: 10.1523/JNEUROSCI.4720-05.2006

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Genetic and Environmental Factors Contributing to Parkinson's Disease: A Case-Control Study in the Cypriot Population

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder affecting a substantial proportion of the elderly Cypriot population. The objective of this study was to evaluate PD risk variants that have been identified previously in Genome Wide Association Studies (GWAS) and to find environmental factors that are predictors for PD onset in the Cypriot population.

Methods: A case-control study was conducted with a total of 235 PD patients and 464 healthy controls of Greek-Cypriot ethnicity. Demographic and lifestyle characteristics, exposure to PD risk factors and clinical data were collected. Moreover, 13 previously GWAS-identified PD risk variants were genotyped. Univariate and multivariate regression analyses examined the association between a number of environmental and genetic factors and PD.

Results: Multivariable regression analysis revealed that exposure to both pesticides and other toxic substances ($P = 0.03$), severe head injury accompanied with fainting ($P = 0.001$), nuts consumption ($P = 0.004$), red meat consumption ($P = 0.02$), and soft drinks consumption ($P = 0.008$) were increasing the risk for PD, whereas cumulative smoking ($P = 0.02$), and fish consumption ($P = 0.02$) were decreasing the risk for PD. Five out of the 13 tested SNPs (rs12185268, rs6599389, rs356220, rs13312, and rs17649553) were confirmed to be nominally significantly associated ($P < 0.05$) with PD risk in the Cypriot population.

Conclusions: Collectively, this case-control study has shed some light on the nature of PD epidemiology in Cyprus, by demonstrating a number of genetic and environmental determinants of PD in the Cypriot population.

Keywords: Parkinson's disease, environmental factors, genetic variants, Cypriot population, observational study, case-control study, epidemiology

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder, characterized by selective loss of dopamine secreting neurons and accumulation of Lewy bodies in the brain and spinal cord (1). It affects 0.3% of the general population and 1% of the population over 60 years old in industrialized countries. The prevalence of the disease is generally higher in Europe and North America compared to South America and Africa (2, 3).

PD is categorized into genetic and sporadic, with the first following Mendelian inheritance and the second being complex. At present, sporadic PD accounts for about 90% of the cases, with the exact pathogenic mechanisms underlying the disease not being completely understood yet (4). However, it is well-known that sporadic PD risk is determined by the complex interplay of genetic and environmental risk factors. Numerous studies and meta-analyses over the last three decades have revealed a number of environmental and genetic risk factors associated with PD risk.

Environmental factors, such as head injury, rural living, pesticides, anxiety/depression, and dairy products intake were positively associated with PD, while physical activity, smoking, coffee consumption, alcohol drinking, smoking, and serum uric acid concentration were reported to be inversely associated with the disease (5, 6).

The genetic component in sporadic PD is currently undisputable. However, the level of heritability of the disease has been debated, with twin studies, family segregation studies and GWAS studies reporting estimates ranging from 6 to 45% (7–9). The heritability value for PD explained by common variants was recently estimated to be 0.21 (10). Currently there are 41 genetic loci that have been associated with PD pathogenesis through 6 large meta-analysis studies (8, 10, 11).

At the moment there is lack of epidemiological data for PD in the Cypriot population. Cyprus is a Mediterranean island and although an isolated population, it is a crossroad between Africa, Europe and Middle East. This made Cyprus a "genetic pool" for transiting populations which gave the genetic signature to the Cypriot population today, characterized by genetic affinity with surrounding Southeast European and Near Eastern populations (12). This renders genetic studies in the Cypriot population informative for the genetically similar populations as well. Characteristic of the genetic admixture and of the peculiarity of the Cypriot population are the geographical clusters of other neurological genetic diseases such as Friedreich ataxia, Huntington disease, and Familial Amyloid Polyneuropathy (13–15). Therefore, the investigation of the epidemiology of other neurological diseases such as PD in the Cypriot population is of particular interest. In addition, it is interesting to investigate which environmental factors are associated with PD in the Cypriot population, a population where some of the PD risk factors are of high prevalence and compare the findings with similar studies involving different populations.

Herein, we aimed to investigate both genetic and environmental determinants of PD in the Cypriot population. Previously published work by our group showed that mitochondrial haplogroups influence the PD risk and age of onset in a gender-specific manner (16). This is the first study exploring the epidemiology of PD in the Cypriot population and will function as a baseline for future studies concerning the etiology as well as the early diagnosis of PD.

METHODS

Study Population and Exposure Assessment

A cohort of 235 PD patients and 464 control subjects were recruited from multiple medical and community centers across Cyprus as described previously (16). Patients were included in the study after clinical diagnosis of PD by a board certified neurologist. Diagnosis was followed by a clinical evaluation, using the UPDRS rating scale by a board certified CING neurologist. Patients that had clinical signs suggestive of Parkinsonian syndromes were excluded.

The 464 ethnically-matched controls were recruited using random cluster sampling across all the districts of Cyprus. Cluster sampling included mailing letters of invitation to residences in randomly selected postal codes as well as visiting randomly selected medical/community centers across Cyprus. Individuals that were $\geq$45 years old and did not suffer from any neurodegenerative disorder or cognitive impairment were invited to participate as controls. All study participants were of Greek-Cypriot nationality.

Epidemiological data from all study participants were collected through a personal interview. The questionnaires consisted of five main sections, which were assessed retrospectively: demographic data, environmental exposure to factors that associated with PD in previous studies (exposure to pesticides and other toxic agents, well water consumption, severe head injury, and intense stress), medical history, lifestyle (diet habits, smoking, alcohol consumption), and anthropometric data (BMI) (5, 6). The questionnaire addressed to the patients, had an additional section covering information about the age of onset, the type of the disease and the symptoms of the disease for each patient.

SNP Selection and Genotyping

Thirteen SNPs that have been associated with PD ($p \leq 5 \times 10^{-8}$) in at least one out of the 5 large GWAS meta-analysis studies for PD in the European population were selected for genotyping (**Supplementary Table 1**) (10, 11, 17–19). The selection criteria for the SNPs were based on the estimates of the association ($0.81 < OR > 1.23$) and on the frequency of the minor allele (MAF $> 5\%$), in order to ensure the maximum statistical power for their investigation. There was an estimation of the power of the study at a value of 0.05 to detect ORs similar to those previously reported in the GWAS, given the allele frequencies observed in the Cypriot population.

DNA was extracted from peripheral blood lymphocytes as described elsewhere (14). SNP genotyping was performed using Taqman genotype assays (Thermo Fisher Scientific). Each assay was carried out using 10 ng genomic DNA in a 5 μl reaction using Taqman Universal PCR Master Mix (ABI). The fluorescence profile was read on an ABI PRISM 7900HT instrument and the results analyzed with Sequence Detection Software (ABI).

Statistical Analysis

Statistical analysis was separated into four parts: descriptive analysis of demographic data, univariate logistic regression analysis, multi-variable logistic regression analysis, and logistic regression for the genetic analysis.

Demographic characteristics of cases and controls were described as frequency and percentage for categorical variables and median and interquartile range (IQR) for continuous variables with a non-normal distribution.

For the comparison of numerical variables between cases and controls the non-parametric Mann Whitney Wilcoxon test was used. For the categorical variables, the chi-square test was employed to compare the frequencies of cases and controls.

Univariate non-adjusted logistic regression analysis was used to test for any association between each variable and PD status. The exposure variables were separated into two large categories: lifestyle characteristics and previously reported exposure risk factors. Lifestyle risk factors included cumulative smoking (cigarettes over lifetime), coffee consumption (cups per month), alcohol intake (glasses per month), food dietary habits (frequency of consumption per month), and indoor and outdoor activities (hours spent per week). Six food categories that are over-represented in the Mediterranean Diet were chosen to construct a new variable called "healthy eating." The Kruskal Wallis non-parametric test was carried out to test whether age of onset differed between the different food consumption categories. Previously reported exposure risk factors include exposure to pesticides, exposure to other toxic and chemical substances, well water drinking, previous severe head injury and exposure to a traumatic experience.

Following all binary logistic regression analyses, the significantly predicting PD risk factors were combined into a multi-variable logistic regression model. Bonferroni correction was applied to account for multiple testing. This enabled us to assess and adjust simultaneously for multiple covariates in relation to a dichotomous outcome; in this case PD.

Trend test was performed for categorical or categorized variables to test if there was a dose-response function between the exposure and the outcome. The level of statistical significance value for the trend analysis test was the 0.05.

All statistical analyses concerning the environmental risk factors were performed using STATA V12 SE statistical software package. SNPStats web-based application (http://bioinfo.iconcologia.net/SNPstats) was used for descriptive statistics of SNPs and assessment of the association of each SNP with PD. Statistical analysis included logistic regression models, adjusted for the age and gender of participants. The log additive model—which indicates how the risk for the disease is modified by each additional minor allele—was chosen to test the association for each SNP with PD.

TABLE 1 | Demographic characteristics of Cypriot PD cases and controls.

Variable		Total	Cases	Controls	*p*-value* (test)
Current age	*N*	691	229	462	**<0.0001** (Wilcoxon)
	Median (IQR)	67 (17)	70 (12)	64.5 (16)	
Age at baseline	*N*	685	226	455	**<0.0001** (Wilcoxon)
	Median (IQR)	64 (15)	62 (16)	64.5 (16)	
No of children	*N*	691	229	462	0.87 (Wilcoxon)
	Median (IQR)	3 (1)	3 (1)	3 (1)	
Gender					
Male	*N* (%)	358 (51.5)	127 (54.5)	231 (50.0)	0.26 (chi-square)
Female	*N* (%)	337 (48.5)	106 (45.5)	231 (50.0)	
BMI (kg)					
Normal weight 20–24.9	*N* (%)	165 (27.3)	64 (34.0)	101 (24.3)	**0.01** (chi-square)
Underweight ≤20	*N* (%)	21 (3.5)	13 (6.9)	8 (1.9)	
Overweight 25–29.9	*N* (%)	252 (41.7)	78 (41.5)	174 (41.8)	
Obesity >30	*N* (%)	166 (27.5)	33 (17.6)	133 (32.0)	
Education level					
Primary school	*N* (%)	281 (40.5)	104 (45.4)	177 (38.0)	0.13 (chi-square)
Secondary school	*N* (%)	94 (13.5)	23 (10.0)	71 (15.3)	
High school	*N* (%)	199 (28.7)	61 (26.6)	138 (29.7)	
Bachelor's degree or higher	*N* (%)	120 (17.3)	41 (17.9)	79 (17.0)	
Retirement					
Not yet	*N* (%)	198 (30.8)	30 (14.1)	168 (39.2)	**<0.001** (chi-square)
Yes	*N* (%)	320 (49.8)	113 (53.1)	207 (48.3)	
Yes, early	*N* (%)	124 (19.3)	70 (32.9)	54 (12.6)	

**P-value nominal significance threshold = 0.05.*
Significant p-values are marked in bold.

TABLE 2 | Lifestyle and previously reported exposure risk factors in Cypriot PD cases and controls.

Variable		Total	Cases	Controls	OR* (95% CI)	*p*-value** (LR)	*p*-trend*** (LR)
CUMULATIVE SMOKING (CIGARETTES OVER LIFETIME)							
Q0: 0	*N* (%)	377 (58.9)	138 (65.4)	239 (55.7)	1.00		**0.02**
Q1: 1–48,000	*N* (%)	53 (8.3)	14 (6.6)	39 (9.1)	0.62 (0.33–1.19)	0.15	
Q2: 48,000–132,000	*N* (%)	50 (7.8)	17 (8.1)	33 (7.7)	0.89 (0.48–1.66)	0.72	
Q3: 132,000–275,000	*N* (%)	53 (8.3)	16 (7.6)	37 (8.6)	0.75 (0.40–1.40)	0.36	
Q4: 275,000–438,000	*N* (%)	54 (8.4)	15 (7.1)	39 (9.1)	0.67 (0.35–1.25)	0.21	
Q5: 438,000–1,940,000	*N* (%)	53 (8.3)	11 (5.2)	42 (9.8)	0.45 (0.23–0.91)	**0.026**	
TOTAL COFFEE CONSUMPTION (CUPS PER MONTH)							
Q1: 0–28	*N* (%)	187 (27.5)	77 (34.1)	110 (24.3)	1.00		**0.009**
Q2: 28–56	*N* (%)	180 (26.5)	57 (25.2)	123 (27.2)	0.66 (0.43–1.02)	0.06	
Q3: 56–84	*N* (%)	168 (24.7)	53 (23.5)	115 (25.4)	0.66 (0.43–1.02)	0.06	
Q4: 84–420	*N* (%)	144 (21.2)	39 (17.3)	105 (23.2)	0.53 (0.33–0.85)	**0.008**	
TOTAL ALCOHOL (GLASSES PER MONTH)							
0	*N* (%)	234 (33.3)	77 (33.0)	153 (32.8)	1.00		0.65
Q1: 0–2.5	*N* (%)	118 (16.8)	43 (18.4)	75 (16.1)	1.14 (0.72–1.81)	0.58	
Q2: 2.5–11.4	*N* (%)	117 (16.6)	36 (15.5)	81 (17.4)	0.88 (0.55–1.43)	0.61	
Q3: 11.4–33.4	*N* (%)	117 (16.6)	41 (17.6)	76 (16.3)	1.07 (0.67–1.71)	0.67	
Q4: 33.4–496	*N* (%)	117 (16.6)	36 (15.5)	81 (17.4)	0.88 (0.55–1.43)	0.61	
HEALTHY EATING							
0	*N* (%)	32 (4.6)	17 (7.3)	11 (2.4)	1		**0.03**
1	*N* (%)	60 (8.5)	18 (7.7)	42 (9.0)	0.28 (0.11–0.71)	**0.007***	
2	*N* (%)	102 (14.5)	36 (15.5)	66 (14.2)	0.35 (0.15–0.83)	0.02*	
3	*N* (%)	142 (20.20)	51 (21.9)	91 (19.5)	0.36 (0.16–0.83)	0.02*	
4	*N* (%)	188 (26.7)	58 (24.9)	130 (27.9)	0.29 (0.13–0.65)	**0.003***	
5	*N* (%)	138 (19.6)	41 (17.6)	97 (20.8)	0.27 (0.12–0.63)	**0.003***	
6	*N* (%)	41 (5.8)	12 (5.2)	29 (6.2)	0.27 (0.10–0.74)	0.01*	

LR, Logistic Regression analysis.
**Univariate non-adjusted Logistic Regression Model.*
***P-value nominal significance threshold = 0.05.*
****Bonferroni adjusted significance threshold = 0.01.*
Significant p-values are marked in bold.

RESULTS

Descriptive Analysis of Demographic Data

A total of 235 PD cases (mean age 66.5 ± 10.5 years, mean age-of-onset 60.4 ± 11.4 years, 54.5% males and 45.5% females) and 464 controls (mean age 65 ± 10.7 years, 50% males and 50% females) were enrolled in this study. PD cases were classified into tremor-dominant (84%) and non-tremor dominant (16%). The prevalence of the most common PD motor and non-motor symptoms of PD cases and their corresponding age at onset are shown in **Supplementary Figure 1**. The demographic characteristics of the study population are listed in **Table 1** and **Supplementary Table 2**. Mann Whitney Wilcoxon test showed that there was a statistically significant difference between the current age of the two groups ($p < 0.0001$), while there was also a significant difference between the age at onset of PD cases and age at recruitment of controls ($p < 0.0001$). Chi square test revealed a statistically significant difference between PD cases and controls for retirement status and BMI ($p < 0.0001$). Logistic regression revealed that BMI was inversely associated with PD, while retirement was positively associated with PD risk after adjusting for current age (**Supplementary Tables 3, 4**).

Univariate Logistic Regression Analysis

Smoking, coffee consumption, alcohol consumption and food dietary habits were tested for their association with PD risk using univariate logistic regression analysis (**Table 2** and **Supplementary Table 5**). There was statistically significant evidence that heavy smokers had about two times less risk to develop PD than non-smokers (OR: 0.45, 95% CI: 0.23–0.91). Coffee consumption was also a predictor for PD in the Cypriot population, with those in the lowest quartile of coffee consumption exhibiting a double risk for PD than participants in the highest quartile (OR: 0.53, 95% CI: 0.33–0.85). This coffee consumption—PD risk inverse association survived Bonferroni correction. Although there was no significant evidence to support that total alcohol consumption affects the risk for PD, heavy wine consumption was inversely associated with PD risk, without accounting for any confounders (OR: 0.54, 95% CI: 0.30–0.96) (**Supplementary Table 5**).

Considering dietary habits, PD cases were consuming significantly more nuts, olives, red meat, carbohydrate rich food, and soft drinks than controls. However, fish consumption was significantly lower in PD cases than controls. PD cases had a significantly lower adherence to "healthy eating" when compared to controls. The associations between food categories and

TABLE 3 | Previously reported exposure risk factors in Cypriot PD cases and controls.

Variable		Total	Cases	Controls	OR* (95% CI)	*p*-value** (LR)	*p*-trend*** (LR)
TOXIC AGENTS							
No toxic agents	*N* (%)	368 (53.3)	108 (47.6)	260 (56.2)	1		**0.001**
Pesticides	*N* (%)	216 (31.3)	71 (31.3)	145 (31.3)	1.18 (0.82–1.7)	0.37	
Other chemical agents	*N* (%)	79 (11.5)	32 (14.1)	47 (10.2)	1.64 (0.99–2.71)	**0.05**	
Both	*N* (%)	27 (3.9)	16 (7.1)	11 (2.4)	3.50 (1.57–7.79)	**0.002**	
WELL WATER CONSUMPTION							
No	*N* (%)	297 (43.2)	95 (42.0)	202 (43.8)	1		0.17
Yes, rarely	*N* (%)	104 (15.1)	24 (10.6)	80 (17.4)	0.64 (0.38–1.07)	0.09	
Yes, systematically	*N* (%)	286 (41.6)	107 (47.4)	179 (38.8)	1.27 (0.90–1.79)	0.17	
SEVERE HEAD INJURY							
No	*N* (%)	489 (71.0)	144 (63.7)	345 (74.5)	1		**0.03**
Yes, with no fainting	*N* (%)	86 (12.5)	31 (13.7)	55 (11.9)	1.35 (0.83–2.18)	0.22	
Yes, with fainting	*N* (%)	114 (87.5)	51 (22.6)	63 (13.6)	1.94 (1.28–2.94)	**0.002**	
INTENSE STRESS/TRAUMATIC EXPERIENCE							
No	*N* (%)	215 (31.5)	79 (35.6)	136 (29.6)	1		0.96
Yes, moderate	*N* (%)	195 (28.6)	46 (20.7)	149 (32.4)	0.53 (0.35-0.82)	**0.004**	
Yes, severe	*N* (%)	272 (39.9)	97 (43.7)	175 (38.0)	0.95 (0.66-1.38)	0.81	

LR, Logistic Regression analysis.
**Univariate non-adjusted Logistic Regression Model.*
***P-value nominal significance threshold = 0.05.*
****Bonferroni adjusted significance threshold = 0.01.*
Significant p-values are marked in bold.

PD risk that remained statistically significant after Bonferroni correction were the following: nuts-PD, red meat-PD, soft drinks-PD and healthy eating-PD. Kruskal Wallis test showed that there was significant difference at the age of onset of PD in Cypriot cases depending on "healthy eating" variable (p = 0.025) (**Supplementary Figure 2**). Physical activity was recorded as indoor and outdoor activities. However, no association was observed between physical activity duration and risk for PD (**Supplementary Table 6**).

Given the positive association previously found between the exposure to pesticides or other chemical substances and PD risk, we evaluated this relationship in the Cypriot population (**Table 3** and **Supplementary Table 7**). Study participants that were exposed to chemical agents had a 64% increased risk for PD (OR: 1.64, 95% CI: 0.99–2.71). The association was considerably stronger when the participants were exposed to pesticides in addition to chemical agents (OR: 3.5, 95% CI: 1.57–7.79). Severe head injury with fainting was also positively associated with PD risk (OR: 1.94, 95% CI: 1.28–2.94). There was evidence supporting that moderate traumatic experience was associated with a 47% decreased risk for PD (OR: 0.53, 95% CI: 0.35–0.82).

Multi-Variable Logistic Regression Analysis

Multivariable Regression Analysis was applied to explore which of the identified predictors for PD in the Cypriot population were independently associated with the disease, even after the adjustment for possible confounders. Therefore, the predictive multivariable model included all 12 variables that exhibited a nominally significant association with PD risk within the unadjusted regression analysis, excluding coffee consumption due to its high collinearity with smoking and adding the age at baseline and gender variables as covariates (**Figure 1** and **Supplementary Table 8**). Multivariate logistic regression Model 1 revealed that the following variables were predictors for PD: fish consumption (OR: 0.39, 95% CI: 0.17–0.87), nuts consumption (OR: 2.74, 95% CI: 1.38–5.45), red meat consumption (OR: 1.92, 95% CI: 1.22–3.33), soft drinks consumption (OR: 2.06, 95% CI: 1.21–3.52), exposure to both pesticides and other toxic substances (OR: 3.28, 95% CI: 1.15–9.36), severe head injury with fainting (OR: 2.42, 95% CI: 1.43–4.09), moderate traumatic experience (OR: 0.41, 95% CI: 0.23–0.72), and heavy smoking (OR: 0.32, 95% CI: 0.13–0.83). Olive consumption, healthy eating, and heavy wine consumption did not preserve nominally statistical association within the multivariate analysis.

Genetic Analysis

Supplementary Tables 9, 10 illustrate the allele and genotype frequencies, respectively, in PD cases and controls, for the 13 SNPs evaluated as well as the allele frequencies for each SNP as reported in the 1000 Genomes project. Deviation from Hardy Weinberg equilibrium was not observed for any of the SNPs in the control subjects (P = 0.15–1). Five out of the 13 SNPs (rs12185268 (OR: 0.69, 95% CI: 0.52–0.90), rs6599389 (OR: 1.50, 95% CI: 1.04–2.16), rs356220 (OR: 1.33, 95% CI: 1.05–1.67), rs13312 (OR: 1.68, 95% CI: 1.23–2.28), and rs17649553 (OR: 0.71, 95% CI: 0.54–0.93) were statistically significantly associated with PD in this study at P less than 0.05 (**Table 4**). Rs12185268 is a missense variant located in *SPPL2C* gene, while rs13312 is a non-coding variant located in the 3 prime untranslated region of *USP24* gene. Rs6599389, rs356220, and

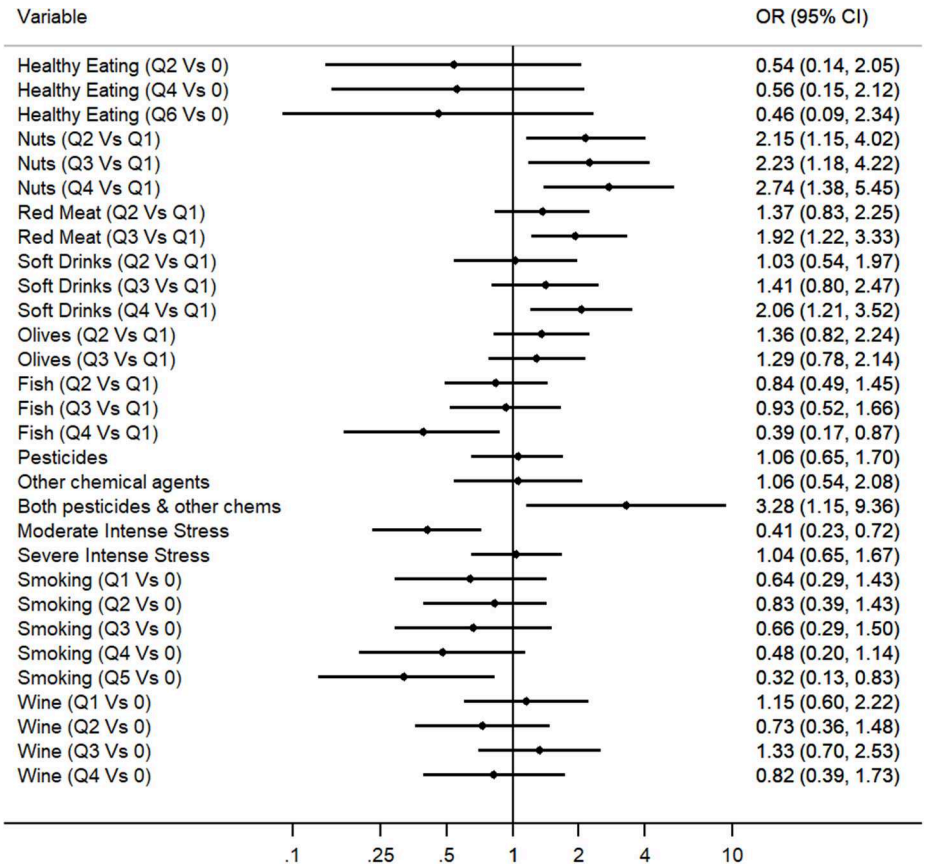

FIGURE 1 | Multivariate logistic regression analysis for the evaluation of the association between environmental factors and PD (OR and 95%CI are represented for each environmental factor).

rs17649553 are intron variants located in *TMEM175, SNCA,* and *MAPT* genes, respectively (http://www.ncbi.nlm.nih.gov/SNP/). Rs823118 (OR: 0.79, 95% CI: 0.62–1.01) and rs356182 (OR: 1.24, 95% CI: 0.98–1.57) SNPs marginally missed the nominal significance level for association with PD risk. The direction of the association of the seven SNPs with PD in the Cypriot population was in line with the direction of the association described in previous GWAS studies.

DISCUSSION

This case-control study confirmed for the first time a number of predictors for PD, related to environmental exposure and genetic risk factors, for the Cypriot population.

The proportion of PD cases that retired early (<65 years old) was almost three times larger than the proportion of controls that retired early. This was in line with an observational cohort study for PD, which showed a hazard ratio of 2.08 for an earlier retirement associated with PD status (20). This shows that the ability to remain in the workforce decreases significantly as the time since onset of the disease increases. Motor and non-motor symptoms make holding an occupation challenging in many psychological and biological aspects for PD patients.

The role of BMI in PD risk is still uncertain, with conflicting results by different epidemiological studies (21, 22). In the current study, we observed a significant inverse association between BMI with PD risk. Weight loss is a frequent early PD symptom as a result of gastrointestinal dysfunction and anorexia (23). In some cases, nutritional complications pre-exist motor-related symptoms. Therefore, one logical interpretation for the inverse association between BMI and PD could be reverse causation. However, this finding is in line with a recent Mendelian randomization study that found a causal association between lifetime exposure to higher BMI and a lower risk for PD (24).

There is compelling evidence that both smoking and coffee consumption are inversely associated with PD risk (25). Our univariate findings regarding cumulative smoking and PD and coffee consumption and PD are consistent with previous findings reporting a protective effect of smoking and coffee consumption for PD. Cumulative smoking was still significantly protective for PD onset when the regression analysis was adjusted for multiple variables. This finding lends support to the hypothesis that biological mechanisms are involved in the smoking-PD relation. One such possible mechanism is the neuroprotective effect of nicotine by modulating the activity of mitochondrial complex I

TABLE 4 | OR and 95% CI for the associations between 13 SNPs and PD risk.

#	SNP	Minor allele	OR (95% CI)*	*P*-value**
1	rs12185268	G	0.69 (0.52–0.90)	**0.006**^
2	rs10513789	G	1.09 (0.82–1.45)	0.57
3	rs6599389	A	1.50 (1.04–2.16)	**0.03**
4	rs356220	T	1.33 (1.05–1.67)	**0.02**
5	rs7617877	A	1.03 (0.80–1.34)	0.80
6	rs17115100	T	1.06 (0.74–1.53)	0.75
7	rs10464059	A	1.13 (0.80–1.60)	0.49
8	rs13312	G	1.68 (1.23–2.28)	**0.001**
9	rs1801582	G	1.08 (0.80–1.46)	0.63
10	rs4837628	C	0.89 (0.69–1.14)	0.36
11	rs823118	C	0.79 (0.62–1.01)	0.056
12	rs356182	G	1.24 (0.98–1.57)	0.076
13	rs17649553	T	0.71 (0.54–0.93)	**0.013**

Logistic Regression Model adjusted for age and gender.
P-value Nominal significance threshold = 0.05.
^Bonferroni adjusted significance threshold = 0.004.*
Significant p-values are marked in bold.

of the respiratory chain and by activating nicotinic acetylcholine receptors in dopaminergic neurons (26).

In the present study, we observed that PD patients had different dietary habits than controls. The multivariable analysis revealed fish consumption as a protective factor and red meat, nuts, and soft drinks consumption as risk factors for the onset of PD. A significant reduction of fish consumption among PD cases was also observed in another retrospective study in the Italian population (27). This protective association is supported by a rat model study proposing that a combination of fish oil with other neuroprotective substances is likely to provide a superior therapeutic advantage in the prevention of oxidative stress-mediated neurodegenerative conditions such as PD (28). This is the first study detecting an increased risk for PD for moderate and heavy soft drinks consumers. A possible explanation could be given by a rat model study which demonstrated that carbonated soft drinks induced oxidative stress and also altered the expression of certain genes associated with brain activity (29). However, soft drinks cover a broad range of drinks, with a large number of components, making it challenging to trace the component that could potentially cause neurodegeneration. The fact that nuts were positively associated with PD risk in the present study could be attributed to the fact that nuts are rich in manganese and iron. The high dietary intake of both iron and manganese demonstrated an almost 2 fold higher risk for PD elsewhere (30). Also nuts have high levels of proteins and fat where organochlorine pesticides are accumulated as it was shown in a toxicology study carried out in India (31). Pesticides inside nuts can accumulate not only from direct pesticide application but also from pesticides concentrated in the soil where nut trees grow. The significant positive association between red meat and PD risk, may be explained by the heme content that may act as a toxin when not digested properly (32). Although there is no study reporting any significant association between red meat by itself and PD risk, it was demonstrated that high intake of animal fat accompanied with low transferrin saturation levels exhibited a 9 fold increased risk for PD when compared to low animal fat intake (33). In addition red meat is rich in saturated fats which increase oxidative stress (34). An unexpected positive association was detected between olives and PD in the univariate analysis. However, this association faded away after adjusting for current age of participants. This can be explained by the fact that older Cypriots tend to consume olives more frequently than younger Cypriots, thus rendering age as confounder in the association.

Exposure to both pesticides and chemical agents were positively associated with PD risk in this case-control study, being consistent with the findings of previous studies (35). One possible interpretation for this positive association could be that the exposure to a variety of environmental toxicants, including pesticides has been associated with differential DNA methylation of genes encoding for enzymes which are key players in cellular redox homeostasis which was found to be involved in PD pathogenesis (36). The results regarding severe head injury with fainting are similar to the pooled results of a meta-analysis study that included 22 studies testing the association between head injury and PD risk (37). Surprisingly, there was a statistically significant protective association detected between moderate intense stress and PD risk. This is possibly a false positive result which could be attributed to the fact that what a PD patient considers as a moderate intense stress differs from what a healthy control considers as a moderate intense stress after the shock of PD diagnosis.

Recent genome wide meta-analysis studies have identified several susceptibility loci for PD (10, 11, 17–19). We have replicated the association of 5 previously reported common variants of small effect size within the *SPPL2C*, *TMEM175*, *SNCA*, *USP24,* and *MAPT* loci for the Cypriot population, even though the analysis was underpowered (8, 11). The significance of the detected associations between the genotyped SNPs and PD risk were weaker in this study when compared to other larger studies from different populations (8, 11, 38). There are two possible scenarios for the failure to replicate the association for the remaining genetic variants. The first explanation could be the restricted power of our study to detect associations with variants of small effect size due to the small sample size and the second could be the fact that the genetic variants identified in previous GWA studies are just proxies for the putative functional variants and therefore population-specific differences allele frequencies and in linkage disequilibrium patterns.

This is the first study exploring both the genetic and environmental determinants for PD in the Cypriot population. Therefore, the results of the current study shed some light regarding understanding the nature of PD epidemiology in the Cypriot population. In addition, given the fact that a large proportion of Cypriots were exposed to risk factors such as pesticides, well water consumption, and intense stress (due to the 1974 war) renders the study essential in understanding which of this factors increase PD in this population and in devising the appropriate prevention strategies. However, the current study has some limitations, including its small sample size which leads to low study power being perhaps its greatest restriction.

Despite, the sample size is generally adequate for very common exposures; it did not provide sufficient power for the detection of expected associations for rare exposures. However, given the fact that Cyprus is a small country and the fact that PD is not a common disease, although it affects a considerable proportion of the elderly population, a larger sample size was almost impossible to recruit. Lastly, due to the observational nature of this study, no inferences could be made regarding the causal nature of the associations identified.

In conclusion, the current study has demonstrated a number of genetic and environmental predictors for PD in the Cypriot population. Multivariable regression analysis revealed that exposure to both pesticides and other toxic substances, severe head injury accompanied with fainting, nuts consumption, red meat consumption, and soft drinks consumption were predisposing factors, whereas cumulative smoking and fish consumption were protective factors for PD risk. The association between rs12185268, rs6599389, rs356220, rs13312, and rs17649553 SNPs and PD risk was replicated in the Cypriot population.

DATA AVAILABILITY STATEMENT

Access to the source data used in this study are available through: https://www.ebi.ac.uk/eva/?eva-study=PRJEB32182.

ETHICS STATEMENT

The study was carried out in accordance with the recommendations of the Cyprus National Bioethics Committee. The protocol was approved by the Cyprus National Bioethics Committee. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AG: (1). Research Project A. Conception, B. Organization, C. Execution, (2). Statistical Analysis A. Design, B. Execution, (3). Manuscript A. Writing of the first draft. CD: (1). Research Project A. Conception, B. Execution, (2). Statistical Analysis A. Design, B. Review and Critique, (3). Manuscript A. Review and Critique. YC and AHe: (1). Research Project A. Conception, B. Execution. EL, PL, EY, MP, KK, and SP: (1). Research Project A. Execution. ML and AHa: (1). Research Project A. Execution. (2). Manuscript A. Review and Critique. EZ-P: (1). Research Project A. Conception, (2). Manuscript A. Review and Critique.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.01047/full#supplementary-material

REFERENCES

1. Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci*. (2005) 28:57–87. doi: 10.1146/annurev.neuro.28.061604.135718
2. Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur J Epidemiol*. (2011) 26:1–58. doi: 10.1007/s10654-011-9581-6
3. Pringsheim T, Jette N, Frolkis A, Steeves TDL. The prevalence of Parkinson's disease: a systematic review and meta-analysis. *Mov Disord*. (2014) 29:1583–90. doi: 10.1002/mds.25945
4. de Lau LML, Breteler MMB. Epidemiology of Parkinson's disease. *Lancet Neurol*. (2006) 5:525–35. doi: 10.1016/S1474-4422(06)70471-9
5. Martino R, Candundo H, Lieshout PV, Shin S, Crispo JA, Barakat-Haddad C. Onset and progression factors in Parkinson's disease: a systematic review. *Neurotoxicology*. (2016) 61:132–41. doi: 10.1016/j.neuro.2016.04.003
6. Bellou V, Belbasis L, Tzoulaki I, Evangelou E, Ioannidis JP. Environmental risk factors and Parkinson's disease: an umbrella review of meta-analyses. *Parkinsonism Relat Disord*. (2016) 23:1–9. doi: 10.1016/j.parkreldis.2015.12.008
7. Wirdefeldt K, Gatz M, Reynolds CA, Prescott CA, Pedersen NL. Heritability of Parkinson disease in Swedish twins: a longitudinal study. *Neurobiol Aging*. (2011) 32:1923.e1–8. doi: 10.1016/j.neurobiolaging.2011.02.017
8. Do CB, Tung JY, Dorfman E, Kiefer AK, Drabant EM, Francke U, et al. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet*. (2011) 7:e1002141. doi: 10.1371/journal.pgen.1002141
9. Moilanen JS, Autere JM, Myllyla VV, Majamaa K. Complex segregation analysis of Parkinson's disease in the Finnish population. *Hum Genet*. (2001) 108:184–9. doi: 10.1007/s004390100470
10. Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, van der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet*. (2017) 49:1511–6. doi: 10.1038/ng.3955
11. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet*. (2014) 46:989–93. doi: 10.1038/ng.3043
12. Heraclides A, Bashiardes E, Fernández-Domínguez E, Bertoncini S, Chimonas M, Christofi V, et al. Y-chromosomal analysis of Greek Cypriots reveals a primarily common pre-Ottoman paternal ancestry with Turkish Cypriots. *PLoS ONE*. (2017) 12:e0179474. doi: 10.1371/journal.pone.0179474
13. Demetriou CA, Heraclides A, Salafori C, Tanteles GA, Christodoulou K, Christou Y, et al. Epidemiology of Huntington disease in Cyprus: a 20-year retrospective study. *Clin Genet*. (2018) 93:656–64. doi: 10.1111/cge.13168
14. Andreou S, Panayiotou E, Michailidou K, Pirpa P, Hadjisavvas A, El Salloukh A, et al. Epidemiology of ATTRV30M neuropathy in Cyprus and the modifier effect of complement C1q on the age of disease onset. *Amyloid*. (2018) 25:220–6. doi: 10.1080/13506129.2018.1534731

15. Zamba-Papanicolaou E, Koutsou P, Daiou C, Gaglia E, Georghiou A, Christodoulou K. High frequency of Friedreich's ataxia carriers in the Paphos district of Cyprus. *Acta Myol*. (2009) 28:24–6.
16. Georgiou A, Demetriou CA, Heraclides A, Christou YP, Leonidou E, Loukaides P, et al. Mitochondrial superclusters influence age of onset of Parkinson's disease in a gender specific manner in the Cypriot population: a case-control study. *PLoS ONE*. (2017) 12:e0183444. doi: 10.1371/journal.pone.0183444
17. International Parkinson's Disease Genomics Consortium, Wellcome Trust Case Control Consortium 2. A two-stage meta-analysis identifies several new loci for Parkinson's disease. *PLoS Genet*. (2011) 7:e1002142. doi: 10.1371/journal.pgen.1002142
18. Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: the PDGene database. *PLoS Genet*. (2012) 8:e1002548. doi: 10.1371/journal.pgen.1002548
19. International Parkinson Disease Genomics Consortium, Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet*. (2011) 377:641–9. doi: 10.1016/S0140-6736(10)62345-8
20. Johnson S, Davis M, Kaltenboeck A, Birnbaum H, Grubb E, Tarrants M, et al. Early retirement and income loss in patients with early and advanced Parkinson's disease. *Appl Health Econ Health Policy*. (2011) 9:367–76. doi: 10.2165/11596900-000000000-00000
21. Wang YL, Wang YT, Li JF, Zhang YZ, Yin HL, Han B. Body mass index and risk of Parkinson's disease: a dose-response meta-analysis of prospective studies. *PLoS ONE*. (2015) 10:e0131778. doi: 10.1371/journal.pone.0131778
22. Hu G, Jousilahti P, Nissinen A, Antikainen R, Kivipelto M, Tuomilehto J. Body mass index and the risk of Parkinson disease. *Neurology*. (2006) 67:1955–9. doi: 10.1212/01.wnl.0000247052.18422.e5
23. Kashihara K. Weight loss in Parkinson's disease. *J Neurol*. (2006) 253 (Suppl. 7):VII38–41. doi: 10.1007/s00415-006-7009-0
24. Noyce AJ, Kia DA, Hemani G, Nicolas A, Price TR, De Pablo-Fernandez E, et al. Estimating the causal influence of body mass index on risk of Parkinson disease: a Mendelian randomisation study. *PLoS Med*. (2017) 14:e1002314. doi: 10.1371/journal.pmed.1002314
25. Breckenridge CB, Berry C, Chang ET, Sielken RL, Mandel JS. Association between Parkinson's disease and cigarette smoking, rural living, well-water consumption, farming and pesticide use: systematic review and meta-analysis. *PLoS ONE*. (2016) 11:e0151841. doi: 10.1371/journal.pone.0151841
26. Quik M, Perez XA, Bordia T. Nicotine as a potential neuroprotective agent for Parkinson's disease. *Mov Disord*. (2012) 27:947–57. doi: 10.1002/mds.25028
27. Cassani E, Barichella M, Ferri V, Pinelli G, Iorio L, Bolliri C, et al. Dietary habits in Parkinson's disease: adherence to Mediterranean diet. *Parkinsonism Relat Disord*. (2017). 42:40–6. doi: 10.1016/j.parkreldis.2017.06.007
28. Denny Joseph KM, Muralidhara. Combined oral supplementation of fish oil and quercetin enhances neuroprotection in a chronic rotenone rat model: relevance to Parkinson's disease. *Neurochem Res*. (2015) 40:894–905. doi: 10.1007/s11064-015-1542-0
29. El-Terras A, Soliman MM, Alkhedaide A, Attia HF, Alharthy A, Banaja AE. Carbonated soft drinks induce oxidative stress and alter the expression of certain genes in the brains of Wistar rats. *Mol med Rep*. (2016) 13:3147–54. doi: 10.3892/mmr.2016.4903
30. Powers KM, Smith-Weller T, Franklin GM, Longstreth WT Jr, Swanson PD, Checkoway H. Parkinson's disease risks associated with dietary iron, manganese, and other nutrient intakes. *Neurology*. (2003) 60:1761–6. doi: 10.1212/01.WNL.0000068021.13945.7F
31. Pandey P, Raizada RB, Srivastava LP. Level of organochlorine pesticide residues in dry fruit nuts. *J Environ Biol*. (2010) 31:705–7.
32. Schipper HM. Heme oxygenase-1: role in brain aging and neurodegeneration. *Exp Gerontol*. (2000) 35:821–30. doi: 10.1016/S0531-5565(00)00148-0
33. Logroscino G, Marder K, Graziano J, Freyer G, Slavkovich V, Lojacono N, et al. Dietary iron, animal fats, and risk of Parkinson's disease. *Mov Disord*. (1998) 13 (Suppl 1):13–6.
34. Peairs AD, Rankin JW, Lee YW. Effects of acute ingestion of different fats on oxidative stress and inflammation in overweight and obese adults. *Nutr J*. (2011) 10:122. doi: 10.1186/1475-2891-10-122
35. Pezzoli G, Cereda E. Exposure to pesticides or solvents and risk of Parkinson disease. *Neurology*. (2013) 80:2035–41. doi: 10.1212/WNL.0b013e318294b3c8
36. Paul KC, Chuang YH, Cockburn M, Bronstein JM, Horvath S, Ritz B. Organophosphate pesticide exposure and differential genome-wide DNA methylation. *Sci Total Environ*. (2018) 645:1135–43. doi: 10.1016/j.scitotenv.2018.07.143
37. Jafari S, Etminan M, Aminzadeh F, Samii A. Head injury and risk of Parkinson disease: a systematic review and meta-analysis. *Mov Disord*. (2013) 28:1222–9. doi: 10.1002/mds.25458
38. Edwards TL, Scott WK, Almonte C, Burt A, Powell EH, Beecham GW, et al. Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. *Ann Hum Genet*. (2010) 74:97–109. doi: 10.1111/j.1469-1809.2009.00560.x

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Exercise-Induced Neuroprotection and Recovery of Motor Function in Animal Models of Parkinson's Disease

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Parkinson's disease (PD) is manifested by progressive motor, autonomic, and cognitive disturbances. Dopamine (DA) synthesizing neurons in the substantia nigra (SN) degenerate, causing a decline in DA level in the striatum that leads to the characteristic movement disorders. A disease-modifying therapy to arrest PD progression remains unattainable with current pharmacotherapies, most of which cause severe side effects and lose their efficacy with time. For this reason, there is a need to seek new therapies supporting the pharmacological treatment of PD. Motor therapy is recommended for pharmacologically treated PD patients as it alleviates the symptoms. Molecular mechanisms behind the beneficial effects of motor therapy are unknown, nor is it known whether such therapy may be neuroprotective in PD patients. Due to obvious limitations, human studies are unlikely to answer these questions; therefore, the use of animal models of PD seems indispensable. Motor therapy in animal models of PD characterized by the loss of dopaminergic neurons has neuroprotective and neuroregenerative effects, and the completeness of neuronal protection may depend on (i) degree of neuronal loss, (ii) duration and intensity of exercise, and (iii) time elapsed between insult and commencing of training. As the physical activity is neuroprotective for dopaminergic neurons, the question arises what is the mechanism of this protective action. A current hypothesis assumes a central role of neurotrophic factors in the neuroprotection of dopaminergic neurons, even though it is still not clear whether increased DA level in the nigrostriatal axis results from neurogenesis of dopaminergic neurons in the SN, recovery of the phenotype of dopaminergic neurons, increased sprouting of the residual dopaminergic axons in the striatum, or generation of local striatal neurons from inhibitory interneurons. In the present review, we discuss studies describing the influence of physical exercise on the PD-like changes manifested in animal models of the disease and focus our interest on the current state of knowledge on the mechanism of neuroprotection induced by physical activity as a supportive therapy in PD.

Keywords: Parkinson's disease, physical activity, neurotrophic factors, neuroplasticity, dopaminergic system

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Due to the frequency of occurrence, it is a serious medical, social, and economic problem. Currently, no proven neuroprotective or disease-modifying treatment is available for PD. Several agents can be used to treat the motor symptoms as well as non-motor symptoms, such as depression, fatigue, sleep disorders, and wakefulness, associated with dopamine (DA) deficiency.

Although pharmacological therapy is the current gold standard in the treatment of PD, recent clinical trials have shown that physical activity alleviates and slows down the development of movement impairments, reduces depression and anxiety, and improves mood state, cognitive function, and sleep quality (1, 2). Recent studies have shown that regular physical activity, such as strength training, walking, flexibility, balance, and aerobic training or dance, adjusted to the severity of the disease and to the current PD patient's state of health, is able to enhance brain plasticity, which plays a key role in improving motor and cognitive functions (3).

Physical activity has been found beneficial for persons with PD; however, there is still no answer as to which type, frequency, or intensity of physical exercise is the most effective in relieving Parkinsonian symptoms. The lack of standardized terminology, protocols, interventions, and outcome measures limits the comparison of data and makes them difficult to interpret.

Aerobic training, aimed mainly to increase cardiovascular capacity, has a beneficial effect on PD patients. Reuter et al. (4) study showed that flexibility and relaxation program, walking, and Nordic walking reduced the pain and improved the quality of life of all patients. Nordic walking proved superior in improving postural stability and gait, and other researchers (5–8) confirmed improvement of movement parameters by this kind of walking. On the other hand, Bello et al. (9) demonstrated the superiority of training on the treadmill compared to overground walking in improving gait and body balance. In yet another study, treadmill training was also found to improve speed, cadence, stride length, and distance walked (10).

A common symptom of PD is muscle weakness. The strength (resistance) training increases muscle mass and bone mineral density, sustains body balance, and thus improves the quality of life of PD individuals. Scandalis et al. (11) showed improved gait function in patients with mild to moderate PD subjected to resistance training. Hirsch et al. (12) confirmed a lower fall risk and a longer independent life of PD individuals in response to resistance and balance exercises.

Improvement in motor function can be seen as specific effect of physical training. However, such activity also preserves or improves cognitive function in PD patients, which suggests training to act as a disease-modifying factor. Physical exercise has been shown to improve performance in verbal fluency tests and to reduce spatial working memory errors in cognitive tests. Cruise et al. (13) showed that a combination of strength and cardiovascular training improved executive function in the course of PD. Tabak et al. (14) and Nocera et al. (15) in small case studies observed improvement in executive function in PD patients after aerobic exercises.

Although the collective evidence supports physical activity as a measure for PD prevention, the mechanism underlying the diminished risk of PD development in physically active persons is still not fully understood (16). Based on animal studies, several mechanisms are believed to explain the effects of physical activity as an adjuvant therapy against PD: increased synthesis and release of neurotrophins (17–20), restoration of the equilibrium between the level and interactions of neurotransmitters (21, 22), increased resistance to oxidative stress (23), reduced inflammatory process in the brain (21, 24, 25), and enhanced synaptogenesis (26), angiogenesis (27), and neurogenesis (28, 29) (**Figure 1**). In the present review, we aimed to describe the influence of physical exercise on PD symptoms in animal models of the disease and report the current state of knowledge concerning the mechanism underlying the neuroprotective effects of physical activity as supportive therapy in PD.

EXERCISE INDUCES RECOVERY OF MOTOR FUNCTION AND NEUROPROTECTION IN ANIMAL MODELS OF PD

Motor Performance

Many studies in animal models of PD showed that various forms of physical activity, differing in type of effort, duration,

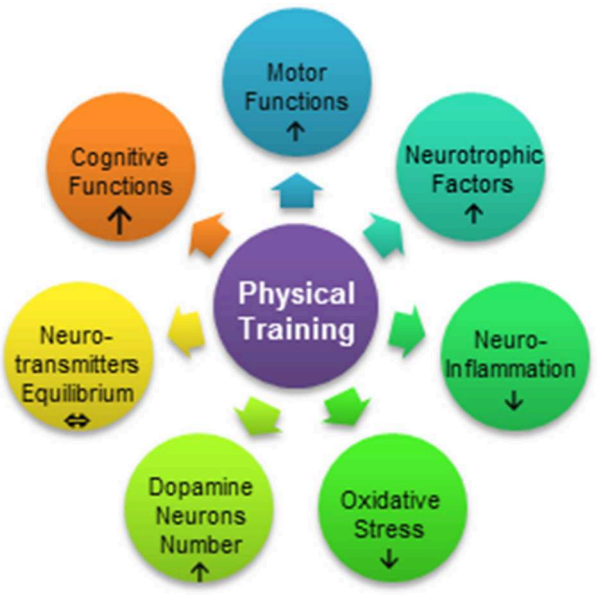

FIGURE 1 | Processes influenced by exercise in Parkinson's disease. Physical training can improve motor function, reduce neuroinflammation, and increase resistance to oxidative stress or reduce the stress level. Motor therapy results in mobilization of neurotrophic factors, protection of dopamine neurons, and restoration of the equilibrium between neurotransmitters such as dopamine and glutamate. Physical exercise may also lead to the enhancement of cognitive functions.

intensity, and starting point with respect to neurotoxic insult, were neuroprotective and suppressed processes involved in PD pathology (30–32).

The two commonly used toxins leading to the degeneration of nigral dopaminergic neurons and, in consequence, to DA depletion in the striatum are 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine [6-OHDA]. The most commonly adopted forms of physical activity in animals are the running wheel, performed voluntarily, and forced treadmill training. There are data proving a reduction of motor performance after administration of PD-inducing neurotoxins (30–33) and a protective effect of physical activity (19, 34–36) against behavioral impairments caused by these neurotoxins in animals. However, the detection and experimental quantification of mouse motor impairments that truthfully mimic the symptoms of PD patients has proved difficult. To detect reliably behavioral deficits using standard mouse motor tests such as the rotarod, inverted grid test, and general locomotor activity monitoring in open field, very large doses of MPTP must be used and behavioral alterations often appear to be transient (32, 37, 38). Movement disorders are only slight in mice performing simple motor tasks, even if the loss of dopaminergic neurons in SN after exposure to MPTP ranges from 60 to 70% (39–41), and this may explain why some researchers report no detectable motor deterioration. Nevertheless, it has been shown that physical activity has both protective (42, 43) and restorative (22, 44, 45) effects on motor parameters such as velocity (46), locomotor activity (42), or body balance and coordination (34, 43).

Several studies report that physical exercise led to motor function improvement in rotarod test in a mouse model of PD (47–51). The MPTP-treated groups that completed a treadmill exercise regimen achieved significantly higher maximal velocity than those in the MPTP sedentary group, although there are data showing no effect of MPTP on the rotarod performance (32, 52). A few reports show that mice treated with MPTP have reduced locomotion; however, there are also studies that report no such changes (53–55) as well as reports in which a restorative effect of endurance exercise on locomotion measured in the open field was noticed (42).

Movement in the upside-down position seems to be a task so complex that it should reveal impairments caused by the loss of dopaminergic neurons; therefore, the inverted grid test was introduced by Tillerson et al. (32). During this test, mouse movement on the underside of the horizontal grid is recorded. Tillerson et al. (32, 56) detected sustained behavioral deficits in MPTP-treated mice up to 28 days post-injection, and these deficits were inversely correlated with striatal DA content and expression of the dopamine transporter (DAT), vesicular monoamine transporter 2 (VMAT2), and tyrosine hydroxylase (TH). The inverted grid test was then used by other researchers (57–63), but none of them demonstrated any correlation between motor performance and the degree of dopaminergic neuron loss. Interestingly, in the study that provides an in-depth analysis of many motor parameters (64), no adverse effect of MPTP treatment was noticed.

The detection and experimental quantification of motor impairments in rodents that truthfully mimic the anomalies of Parkinsonian patients proved difficult either due to inadequate behavioral tests or—very likely—because motor impairment takes another form in these animals. So, at present, despite the known ability of physical exercise to promote motoric improvement, our knowledge regarding the most beneficial form, duration, intensity, and frequency of exercise is still insufficient.

Protection of Dopaminergic Neurons

Depending on when physical training is commenced with respect to neurotoxic insult, one may examine (i) the preventive role of exercise against PD once such insult follows training; (ii) the modification of the course of disease, when the chronic treatment is paralleled with training; and (iii) the neuroregenerative effect of exercise when physical training is applied post-insult.

The neuroprotective role of exercise against the loss of dopaminergic neurons was studied by Gerecke et al. (65), where voluntary physical activity on the running wheel was applied before acute administration of MPTP. The authors determined the critical duration of voluntary exercise necessary for neuroprotection of dopaminergic neurons. They found that 1 month of training provided no neuroprotection, 2 months of training conferred partial protection, and only the first 3 months of training prevented any loss of substantia nigra pars compacta (SNpc) dopaminergic neurons by subsequent neurotoxin treatment. However, beside duration of the training, the distance covered also turned out to be essential. It was found that the longer distance run on the wheel, the lesser loss of DA neuron in the 3-month training group, so only mice that had run the longest distance were completely protected against MPTP toxicity.

The effects of ongoing training on the progression of PD were modeled with daily treadmill running during chronic neurotoxin treatment realized with 10 injections of MPTP spread over 5 weeks. Such chronic MPTP treatment leads to permanent, lasting at least 6 months, neurological deficits resembling, though incompletely, PD. This is unlike acute and subacute MPTP treatments, after which neurological deficits and behavioral changes soon wane spontaneously (65).

Ahmad et al. (66) using this chronic mouse model proved that 10- and 18-week-long treadmill training, started 1 week before commencing MPTP treatment, reduced loss of dopaminergic neurons in the ventral tegmental area (VTA). It should be noted, though, that this model encompasses both parallel application of neurotoxin and physical training as well as post-insult training. Somewhat surprisingly, the number of DA neurons was greater following 18 weeks of exercise training, than 10, which may prove that either new DA neurons appeared and/or some DA neurons that lost their phenotype regained it during subsequent 8 weeks.

Pothakos et al. (42), using the same model, started treadmill training 1 week before MPTP treatment, continued it during intoxication lasting 5 weeks, and over the subsequent 8–12 weeks. They did not observe either the recovery of striatal DA or preservation of TH positive neurons in the SNpc in exercising MPTP-treated mice. It may be important that different doses of MPTP have been used in these studies—12.5 vs. 25 mg/kg

of MPTP per injection. The importance of dose was confirmed by Lau et al. (19). In the same chronic model of PD with 18-week treadmill training, which was started 1 week before MPTP intoxication, they observed nearly complete preservation of TH immunopositive neurons in the SNpc and the level of striatal DA comparable to that in control mice. The importance of MPTP dosing was reflected in the loss of 55% of TH positive neurons observed in the study of Lau et al. (19) who used 15 mg MPTP/kg/injection and a 72% loss in the study of Pothakos et al. (42) who applied 25 mg/kg/injection.

Two studies that used the protocol described above delivered contrasting results: Pothakos et al. (42) did not observe either the recovery of striatal DA or preservation of TH positive neurons in the SNpc in exercising MPTP-treated mice, whereas Lau et al. (19) found nearly complete preservation of TH immunopositive neurons in the SNpc and the level of striatal DA comparable to that in control mice. The possible explanation of this discrepancy may be the dose of MPTP, which, in Pothakos et al. study, is twice as big as in Lau et al. study. The difference in MPTP dose was reflected in the 72% loss of nigral DA neurons in the former vs. 55% in the latter study.

Studies on the beneficial effects of exertion applied after neurotoxin application show varying results: from no effect to partial or complete preservation of the number of TH-positive nigral neurons. Aerobic training lasting 4 weeks and started after 5 weeks of treatment with 25 mg MPTP/kg/injection slightly raised nigrostriatal TH and DA levels as compared with sedentary MPTP-injected mice (67). Kintz et al. (22) found that training lasting 37 days, which started 5 days after acute dosing with MPTP, did not return the level of striatal DA reduced by the neurotoxin. Zhao et al. (68) found that the number of nigral dopaminergic neurons returned almost to that observed in control mice after 4 weeks of vibration training following 1 week of MPTP treatment and was also significantly greater than that in sedentary MPTP-treated animals. This was accompanied by similar changes in the striatal DA level. In another study (69), it was found that 8 weeks of progressive treadmill that started 2 weeks after chronic high-dose (25 mg/kg/injection) MPTP administration caused nearly complete restoration of TH-positive neurons in the SNpc, as well as similar recovery of TH and DAT in MPTP-treated exercising mice.

Increased number of dopaminergic neurons observed in neurotoxin-treated animals after physical exercise could be the result of better protection of neurons or neurogenesis. In a study performed by Jang et al. (45), mice were intraperitoneally injected with 25 mg/kg MPTP daily for 1 week. Training on the treadmill lasted 6 weeks and was applied 4 weeks after the last MPTP dose. This training induced neurogenesis in MPTP-treated mice, evidenced by an increased number of bromodeoxyuridine (BrdU)-positive neurons, and attenuated the loss of dopaminergic neurons as manifested by higher levels of TH and DAT. The authors also showed enhanced autophagy, exemplified by changes in autophagy-related protein levels (e.g., microtubule-associated protein 1A/1B-light chain 3—MAP1LC3, nucleoporin p62—p62, Beclin 1, Beclin 2 (Bcl-2)/adenovirus E1B-19 kDa interacting protein—BNIP3, lysosome-associated membrane protein 2—LAMP2, cathepsin L and transcription factor EB—TFEB), and augmented antioxidant capability (e.g., increased level of superoxide dismutase-1—SOD-1, catalase, glutathione peroxidase 1—GPX1, heme oxygenase-1—HO-1, and DJ1) as compared with the results obtained in the MPTP group with no treadmill training.

Also, in a rat model of PD induced by 6-OHDA injections, significant preservation of TH-immunopositive neurons in the SNpc and fibers in the striatum was observed after 4 weeks of treadmill training applied 24 h post-neurotoxin insult (70).

The neuroprotective potency even of a late (i.e., post-neurotoxic insult) physical training was recently illustrated by our study (71), where training of the same duration and intensity attenuated neuronal loss to the same degree, both when commenced before or after chronic MPTP treatment.

Although most reports prove that physical activity positively affects motor functions (19, 34, 44, 47–50, 72) and increases the level of DA in the striatum (19, 73), there are also studies reporting the lack of effects of physical exercise on these parameters (22, 52, 53, 55). For example, Gerecke et al. (65) have found that, even though 3 months of training completely protected dopaminergic neurons against MPTP-induced neurotoxicity, the level of DA and its metabolites in the striatum was significantly lower compared to controls. In order to account for these discrepant findings, the authors hypothesize that uptake of 1-methyl-4-phenylpyridinium (MPP^+) by DAT generates the formation of hydrogen peroxide and nitrosylated proteins. These compounds damage the dopaminergic terminals but not the cell body. It is also possible that MPP^+ alone lowers the ability of dopaminergic neurons to transport DA into striatal terminals leading to decreased DA level.

REGULATION OF DOPAMINERGIC AND GLUTAMATERGIC TRANSMISSION *VIA* PHYSICAL ACTIVITY

Degeneration of dopaminergic neurons in the SNpc and striatal loss of axonal terminals are key pathological features of PD in humans (74–76) and in toxin-induced animal models (19, 42, 71, 77). DA deficiency in PD also leads to loss of dendritic spines within the striatum, which results in motor impairments (78). Two different types of dopaminergic activity can be observed in the striatum: a phasic, brief high-amplitude increase of DA release that acts at the synaptic space through the low affinity D1 receptor (D1R) and a tonic DA release of low amplitude that acts through the high-affinity D2 receptors (D2R) located in the extrasynaptic space. The striatum is functionally subdivided into ventral and dorsal areas, which participate in different aspects of motor control (74). The dorsal striatum is mainly composed of two subpopulations of medium spiny neurons (MSNs): DA D1 receptor-expressing MSNs that constitute the striatonigral or direct pathway (dMSNs) and DA D2 receptor-expressing MSNs that constitute the striatopallidal or indirect pathway (iMSNs). It has been suggested that each pathway has a different role in motor control, with dMSNs being involved in the main aspects of motor control, including motor program selection and activation, and iMSNs in selection and activation of a context-specific motor

programs based on the integration of motivational/emotional signaling with sensory-motor inputs (79).

Toy et al. (77) have found a decrease in dendritic spine density in both D1R and D2R containing MSNs after acute MPTP administration and an increase in dendritic spine density and arborization in MSNs of both pathways after 30 days of intensive treadmill exercise. Recovery of dendritic spines was associated with increased expression of post-synaptic density protein 95 (PSD-95) and of presynaptic synaptophysin, leading to increased synaptogenesis in the dorsolateral striatum and improvement in motor performance (77).

The deficiency of DA leads to structural and functional changes in MSNs. These changes usually entail increased glutamatergic projection and hyper-excitability of the indirect pathway (D2R-iMSNs) (80). The prolonged elevated level of glutamate in the intercellular space results in longer depolarization and in disturbances of ionic homeostasis that elicits excitotoxicity and, in consequence, cell death. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid receptor (AMPAR), a fast-acting ionotropic glutamate receptor, plays a critical role in these processes. AMPARs are tetrameric channels composed of various combinations of four glutamate receptor subunits, GluA1–GluA4. In particular, the presence or absence of GluA2 decides about important electrophysiological channel properties, including calcium (Ca2+) permeability. Reduced level of GluA2 in AMPAR channels may lead to increased permeability for calcium ions.

Alterations in AMPARs expression have also been noted in animal models of PD characterized by a reduced level of DA. Kintz et al. (22) have noticed that MPTP treatment alone or combined with exercise evoked changes in AMPAR level limited to the D2R-iMSNs striatopallidal pathway. They observed an increase in the level of AMPARs lacking GluA2 in MPTP-treated mice. Increased expression of the GluA2 subunit of the AMPAR in animals that started to exercise after acute MPTP administration suggested the restoration of normal AMPAR subunit expression. Enhanced expression of GluA2-lacking AMPAR possibly potentiates glutamatergic signaling, which leads to hyperexcitability of the striatopallidal projection pathway observed as a consequence of DA depletion (81). Exercise increased the presence of GluA2 subunit in AMPARs in MPTP-treated mice. This could decrease Ca^{2+} influx and diminish glutamatergic drive leading to reduced glutamatergic projection and thus increased survival of dopaminergic neurons.

The hypothesis of increased glutamatergic projection in the course of PD and reduced glutamatergic tonus in response to physical activity has been strengthened by Scone et al. (21), who showed that while levels of vesicular glutamate transporter 1 (VGLUT1) and glutamate transporter-1 (GLT-1) were elevated after MPTP administration, the level of these transporters was decreased following physical activity, restoring glutamate homeostasis in treated mice. Fisher et al. (44) analyzed the neuroprotective effect of 30 days of treadmill training on proportions between dopaminergic and glutamatergic projection in C57 BL/6J mice acutely administered with MPTP. The treadmill training was started 4 days after MPTP or saline treatment. The MPTP exercise group demonstrated significantly reduced DAT immunoreactivity, higher expression of D2R mRNA, and a significant decrease in the density of glutamate terminals compared to the MPTP sedentary group. An increase in the density of nerve terminal glutamate immunolabeling, characteristic for MPTP lesioning, may, therefore, reflect a decrease in the extracellular levels of striatal glutamate. Consequently, the effect of exercise in an MPTP-lesioned brain may be the increased release of glutamate at the synapse, which may alter DA receptor subtype expression or/and function of medium spiny neurons.

EXERCISE MOBILIZES NEUROTROPHIC FACTORS

Post-mortem examinations of PD brain in human and animal models demonstrate a decreased level of neurotrophic factors (NTFs) in the nigrostriatal pathway (82–84), although this reduction could be partially due to the loss of SNpc dopaminergic neurons, which specifically express one of the NTFs, the brain-derived neurotrophic factor (BDNF). Decreased efficiency of neurotrophic factors, such as BDNF, nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) in PD may be connected with a reduced level of cyclic nucleotides. Such reduction leads to dysregulation of transcription of NFT encoding genes, mediated by the cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) (85–87).

NTFs are endogenous proteins that promote differentiation, maintenance, function, and plasticity of the nervous system and allow neurons to survive and repair after injury (88). Therefore, NTFs may serve as potential therapeutic agents in the treatment of neurodegenerative diseases including PD. For example, BDNF, mesencephalic astrocyte-derived neurotrophic factor (MANF), glial cell line-derived neurotrophic factor (GDNF), and cerebral dopamine neurotrophic factor (CDNF) have been shown to be neuroprotective and neurorestorative toward damaged dopaminergic neurons in cell cultures and in various PD animal models (89). *In vivo*, NTFs induce survival of nigrostriatal DA cell bodies and fibers improving motor performance compromised in parkinsonian animals.

The best known trophic factors that protect dopaminergic neurons against oxidative stress are BDNF and GDNF. Initially, the potential therapeutic role of BDNF and GDNF in dopaminergic neurons was found in *in vitro* studies (31, 89, 90). BDNF is widely and abundantly expressed in the brain and significantly involved in several aspects of neuronal development and maturation, plasticity, and recovery mechanisms (91, 92). BDNF also supports the survival of several types of neurons, including mesencephalic dopaminergic, septal cholinergic, and striatal gamma-aminobutyric-acid-releasing (GABAergic) neurons. Binding of BDNF to the high-affinity tropomyosin-related kinase B (TrkB) receptor leads to phosphorylation of TrkB and activation of the three essential downstream intracellular signaling cascades within neuronal somata: phospholipase C-γ (PLCγ), phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), and mitogen-activated protein kinase/extracellular

signal-related kinase (MAPK/ERK) pathways. Furthermore, BDNF may activate the CREB transcription factor and CREB-binding protein (CBP) and regulate expression of genes encoding proteins involved in stress resistance and cell survival and even neural plasticity (93).

GDNF, in turn, is believed to be the most important neurotrophic factor in the nigrostriatal dopaminergic system and therefore is considered to have therapeutic potential for neuroprotective and regenerative interventions in PD (88). GDNF promotes the survival and maturation of dopaminergic neurons and increases their high-affinity DA uptake (89). It exerts similar effects on motor, adrenergic, parasympathetic, enteric, and somatic sensory neurons (94). GDNF binds with high affinity to glycosylphosphatidylinositol-linked receptor α1 (GFRα1), which is highly expressed in midbrain dopaminergic neurons when measured at mRNA and protein levels. The complex of GDNF and GFRα1, in turn, recruits a transmembrane rearranged during transfection (Ret) receptor and triggers downstream signaling, which controls mitochondrial morphology and complex I activity in dopaminergic neurons (94). GDNF, after binding to its receptor, activates signaling pathways leading to stimulation of dopaminergic neuron excitability, inhibition of DAT activity, and stimulation of TH phosphorylation (95).

There is increasing evidence that NTFs are critical for exercise-induced neuroprotection. The study of Lau et al. (19) has shown that the exercise-induced recovery of cell number and motor behavior in the chronic MPTP mouse model of PD was associated with an improved mitochondrial function and an increase in the brain region-specific levels of BDNF and GDNF. Furthermore, Zhao et al. (68) demonstrated that in MPTP mice, 4 weeks of vibration training almost completely restored dopaminergic neurons in the SN, lost due to MPTP treatment, and DA levels in the striatum, and significantly increased the level of BDNF in the striatum. The authors postulated that long-lasting vibration training could protect dopaminergic neurons from MPTP-induced damage probably by upregulating BDNF. In turn, Gerecke et al. (20), using $BDNF^{+/-}$ mice, showed that exercise was not able to protect dopaminergic neurons from MPTP-induced neurodegeneration.

In another study by Tajiri et al. (70), daily forced treadmill training was used in the 6-OHDA acute rat model of PD. In the 6-OHDA training group, behavioral recovery in the cylinder test and a significant decrease in the number of amphetamine-induced rotations were observed. This was accompanied by the preservation of dopaminergic fibers in the striatum and neurons in the SNpc. In addition, BrdU/doublecortin (Dcx) co-staining revealed enhanced proliferation and migration of neural progenitor cells from the subventricular zone (SVZ) toward the injection site. At the same time, BDNF and GDNF levels were upregulated in the striatum. Physical training in animal models of PD, beside neuroprotection of the dopaminergic system, may enhance neurogenesis and progenitor cell migration through upregulating BDNF-TrkB and GDNF-Ret signaling, which can, in turn, stimulate certain signaling cascades, including those that activate the PI3K/Akt pathway or the extracellular signal-regulated kinases 1 and 2 (ERK1/2) cascade. The PI3K/Akt signaling pathway transmits anti-apoptotic signals stemming from neurotrophins. This pathway activates transcription factors such as CREB and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), which trigger the expression of genes involved in cell survival, such as Bcl-2 and other inhibitors of apoptosis. It also suppresses the expression of pro-apoptotic genes, such as the Bcl-2-associated death promoter (BAD) and Forkhead box (FOX) transcription factors (96). Similarly, the ERK1/2 signaling cascade induces anti-apoptotic genes such as those encoding Bcl-2 and the transcription factor CREB. ERK1/2 also mediates neuritic outgrowth induced by neurotrophic factors (97) and phosphorylation of TH, which increases its enzymatic activity, thus enhancing DA synthesis (98).

Additionally, physical activity, through the induction of neurotrophin signaling pathways, affects synaptic plasticity in MPTP mice. In the study of Zhu et al. (99), the authors have demonstrated that long-term potentiation (LTP) impairment in the MPTP model was due to the decrease in hippocampal BDNF level. Both induction of endogenous BDNF synthesis by memantine or application of exogenous BDNF restored the LTP deficit in the MPTP model. In addition to the effects of memantine on LTP, MPTP-enhanced long-term depression (LTD) was also reversed by memantine administration. These data suggest that compounds that can activate BDNF synthesis have the potential to protect memory in PD.

The evidence that exercise, through regulation of growth factors, secures successful brain function was demonstrated in several other studies (100–104). Also in our study (71), the results suggest that 10 weeks of training on the treadmill, no matter if started before or after PD induction, have a protective effect against dopaminergic neuron degeneration in the chronic MPTP mouse model of PD. Neuroprotective properties of exercise were most likely associated with increased expression of endogenous NTFs and reduced inflammation in the brain. Although the actual sequence of events needs to be discerned, it is likely that NTFs protect neurons against degeneration and in this way prevent the inflammatory response. However, the other way is also possible, i.e., that the strength of inflammation is regulated by the neurotrophin signaling pathways irrespective of neurodegeneration. It is likely that both ways operate when training accompanies neurotoxic assault and also when it is applied after such assault. In summary, these studies underscore the view that physical activity remains neuroprotective even during the advanced stage of PD and therefore provide strong support for starting and continuing physical activity at any point of the disease.

PHYSICAL TRAINING MODULATES NEUROINFLAMMATORY MECHANISMS

Although the death of dopaminergic neurons is a well-characterized pathological feature of PD, the primary cause of the disease is still not clear. Since the first observations of reactive microglia and astrocytes in *post-mortem* brain samples of PD patients were made (105, 106), numerous studies have proposed a role of glial cells in the neuropathology of PD (107–109). Furthermore, infiltration of cells of the peripheral

immune system into the brain is also likely to play a role in the pathomechanism of PD (110, 111). It is not clear whether inflammation is a primary cause of PD or a secondary event, that is, the consequence of neuronal death. Short-lasting inflammatory reactions may induce NTFs (112, 113) and may protect neurons against reactive oxygen species (ROS) (114) and glutamate neuronal hyperexcitability (115) but chronic inflammation usually leads to degeneration and neuronal damage (113, 116, 117).

Neuroinflammation means activation of microglia and reactive astrocytes, which, in turn, produce cytokines, chemokines, reactive oxygen, and nitrogen species, secondary messengers, prostaglandins, and protein complement cascades (118). Elevated levels of interleukin-6 (IL-6) acting as pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and nitric oxide synthase (NOS), found in the SN, putamen, as well as in the cerebrospinal fluid (CSF) and serum of PD patients suggest that, in PD, glial cells acquire a pro-inflammatory phenotype (119).

Both the pro-inflammatory cytokines and their receptors play a role in the pathogenesis of PD. A glial reaction in the brain involving astrocytes and microglia have been described in several toxin-induced animal models of PD (21, 46, 120, 121). Loss of dopaminergic neurons is associated with activated microglia (122–124), which, in turn, activates astrocytes. Increasing evidence suggests that the CD200 protein and its receptor, CD200R, play a critical role during microglia activation. Deficits in the CD200–CD200R pathway exacerbate microglial activation and degeneration of dopaminergic neurons (125).

Liddelow et al. (126) have shown in *in vitro* and *in vivo* studies that activated microglia stimulate, in turn, astrocytes by secreting interleukin-1 alpha (IL-1α), TNFα, and complement component 1q (C1q). Lofrumento et al. (109) have observed a significant increase in IL-1β, TNF-α, and IL-6 mRNA expression level and an increase in both mRNA and protein levels of their respective receptors IL-1RI (IL-1 receptor type I), TNF-αRI, and IL-6Rα in the SN of MPTP-treated animals in comparison with untreated mice.

Astrogliosis is characterized mainly by an increase in the number and size of astrocytes and increased expression of the glial fibrillary acidic protein (GFAP). Due to pro-inflammatory activation, astrocytes lose the ability to promote neuronal survival, neurite outgrowth, synaptogenesis, and phagocytosis (127). Astrogliosis has been confirmed during PD (35, 128). Sconce et al. (21) have shown an increase in GFAP immunoreactivity and a reduced ratio of phosphorylated to non-phosphorylated component 3 of the nuclear factor of activated T-cells (NFATc3) within the SN in MPTP mice.

The neuroprotective effect of physical activity has been linked to the prevention and modulation of the inflammatory process (129). Recent evidence suggests that physical activity, in addition to modifying the cardiovascular, muscular, and hormonal systems, also affects the immune system, which can lead to the overall anti-inflammatory effect in the whole body (130), including attenuation of the inflammatory processes in the brain in the course of neurodegenerative diseases.

Sconce et al. (21) have reported that running wheel exercise affects behavioral deficits, as well as dopaminergic, glutamatergic, and inflammatory biomarkers in a progressive MPTP mouse model of PD. The authors noticed recovery of motor abilities, increased VMAT2 expression, decreased glycosylated-DAT expression, reduced levels of VGLUT1 and GLT-1, and lower levels of the inflammatory marker, component 3 of the nuclear factor of activated T-cells (NFATc3), and of the astrocytic marker, GFAP, in MPTP/exercised mice as compared to MPTP mice without exercise. The anti-inflammatory effect of different types of physical activity has been verified by Goes et al. (24). The authors found that 4-week swimming training alleviated cognitive and motor impairment, and prevented both the increase of ROS and IL-1β levels and inhibition of glutathione peroxidase (GPx) glutathione *S*-transferase (GST) and glutathione reductase (GR) activities. This training also restored DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) activity levels in the striatum of mice administered with 6-OHDA (24).

Recent studies have suggested that interaction between alpha-synuclein (α-syn) and toll-like receptor 2 (TLR2) may play a critical role in the onset of neuroinflammation (131). Jang et al. (132) observed in mice that 8-week treadmill training started after completion of chronic MPTP treatment eliminated motor coordination deficits, increased nigrostriatal TH level, and diminished expression of α-syn in the striatum. This, in consequence, led to downregulation of TLR2 signaling molecules such as myeloid differentiation primary response 88 (MyD88), tumor necrosis factor receptor-associated factor 6 (TRAF6), and phosphorylated transforming growth factor-β-activated protein kinase 1 (p-TAK1). What was interesting, physical activity significantly decreased GFAP level and increased the level of phosphorylated NFATc3, thus reducing the susceptibility of dopaminergic neurons to apoptosis.

In another study (71), the immunohistochemical staining and ELISA assay against GFAP showed an increased number of astrocytes and elevated GFAP concentration in SNpc and VTA in MPTP sedentary mice. On the other hand, treadmill training caused reduced GFAP concentration in the MPTP-trained group, comparable to the concentration of GFAP observed in the control groups. Furthermore, staining against CD11b and ionized calcium-binding adapter molecule 1 (Iba1), the markers of microglia, also showed higher intensity in the SNpc and VTA of MPTP mice without treadmill training compared with results obtained in controls and the MPTP group with treadmill training (71).

Both subtypes of glial cells, astrocytes and microglia, may be activated in two different ways, resulting in a pro-inflammatory (classical M1 activation) or anti-inflammatory (alternative M2 activation) formation of response. In the latter case, stimulated microglia show increased expression of cytokines recognized as an anti-inflammatory, such as interleukin-10 (IL-10), transforming growth factor beta (TGFβ), insulin growth factor 1 (IGF-1), NGF, and BDNF (133). Astrocytes, similarly to microglia, secrete anti-inflammatory compounds, among them neurotrophic factors such as GDNF, BDNF, and MANF, which

revive injured dopaminergic neurons and uphold their survival (134, 135).

It is likely that physical training applied in MPTP mice not only reduces M1-type pro-inflammatory glial activation but also promotes M2-type neuroprotective activation of microglia. The increased synthesis of trophic factors observed after prolonged physical training could favor the neuroprotective pathway of microglia activation. In consequence, dopaminergic neurons protected by neurotrophins do not degenerate and thus do not send signals that mobilize the pro-inflammatory response and proliferation of glial cells. Training may reduce the reactivity of microglia and mitigate inflammation by altering multiple metabolic and transcriptional processes (136). For instance, it may inhibit the activity of glycogen synthase kinase 3 (GSK-3), which is a major regulator of the balance between the pro- and anti-inflammatory mediators in immune cells, including microglia (137). GSK-3 stimulates activated microglia to release pro-inflammatory cytokines such as IL-1β, IL-6, and TNFα, and inhibits the release of anti-inflammatory cytokines such as IL-10 (138–140). The way the exercise affects GSK-3 activity may rely on the activation of some extracellular factors, including those mediated by BDNF (141, 142) and GDNF (143, 144), which are known to inhibit GSK-3.

PHYSICAL TRAINING ATTENUATES MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS

It has long been recognized that oxidative stress may be important in the etiology of a variety of late-onset neurodegenerative diseases including PD (145–147). Oxidative stress is connected with the production of ROS, which show strong oxidizing properties. Although ROS at physiological concentrations play a pivotal role in several cellular signaling pathways such as cell cycle regulation, phagocytosis, and enzyme activation, excessive generation of ROS leads to several harmful effects including damage to DNA, lipids, and proteins (148).

In the PD brain, the nigral polyunsaturated free fatty acid level is reduced, while the nigral level of lipid peroxidation markers (malondialdehyde and 4-hydroxynonenal) is elevated (149). Also, protein oxidative damage is evidenced by the presence of protein carbonyls (150). Some results suggest that reactive nitrogen species contribute to PD etiology due to their role played in nitration and nitrosylation of certain proteins (151). Oxidative stress leading to cell death may occur in the SNpc because of (i) augmented production of H_2O_2 due to increased DA turnover, (ii) reduced brain capacity to eliminate H_2O_2 because of glutathione (GSH) deficiency, or (iii) promotion of $^{\bullet}OH$ formation caused by elevated level of the reactive iron (152, 153). Synthesis of neuronal GSH requires delivery of GSH precursors, which mainly arise from extracellular cleavage of astrocyte-derived GSH. Lower nigral levels of GSH have been found in *post-mortem* PD patient brains when compared with age-matched controls. Diminished GSH release from astrocytes may be caused by depletion of reduced GSH in astrocytes, which in turn is caused by increased oxidant production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) (154).

Mitochondria are a key point for programmed cell death (PCD) where apoptosis may be triggered by several factors produced as a result of intracellular stress, such as ROS and calcium ions (Ca^{2+}) overload (155). It has been reported that, due to oxidative stress, there are more deletions in mitochondrial DNA of the surviving nigral dopaminergic neurons of PD patients (156, 157). The hypothesis of mitochondrial dysfunction in PD was strengthened after the discovery of mutations in several genes encoding mitochondrial proteins that give rise to a familial form of PD (e.g., *PINK1* and *PARK2, DJ-1*) (158).

The involvement of mitochondrial dysfunction and mitochondrial ROS production has been demonstrated also in experimental animal models using either toxin (MPTP) or genetically altered mice. The *post-mortem* examination of SNpc in PD patients showed a significant increase in the H-subunit and a significant reduction in the L-subunit of ferritin, which was associated with increased iron concentration, ROS overproduction, and neuronal death (159). A reduction in complex I activity has been demonstrated in the SN, lymphocytes, and platelets of PD patients (160). Moreover, increased oxidative stress has been detected in both the rotenone and MPTP-induced toxin models and in genetic models of PD (160, 161). Studies in animals have indicated that MPP^+, the active metabolite of MPTP, and rotenone inhibit ATP synthesis by blocking electron transport. This blockade is due to binding to complex I. MPP^+, similarly to rotenone, produces superoxide anions in electron transport particles, which supports the view that MPP^+ is primarily a mitochondrial toxin (162). The neurotoxic effects of MPP^+ can be effectively prevented by antioxidants, indicating that the neurotoxicity of this compound is due to oxidative stress (163).

Many studies demonstrate a positive effect of physical exercise on inhibition of oxidative stress (47, 69, 164–172). Using a chronic mouse model of PD, Patki, and Lau (173) revealed that 18-week treadmill training inhibited cytochrome c release, elevated levels of p53, and upregulated the expression of mitochondrial transcription factor A (THAM) and of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), which are known to be associated with mitochondrial dysfunction and loss of dopaminergic neurons.

Koo et al. (69, 174) demonstrated, in a MPTP/probenecid-induced mouse model of PD, that treadmill training improved mitochondrial function and promoted autophagy *via* the sirtuin-1 (SIRT1) signaling pathway causing α-syn level to decrease. This subsequently attenuated the loss of dopaminergic neurons due to reducing apoptotic cell death mediated by α-syn. Most importantly, training increased expression of SIRT1 that, in turn, enhanced mitochondrial biogenesis and reduced oxidative stress by activating PGC-1α. Furthermore, activation of SIRT1-activated microtubule-associated protein 1 light chain 3 (MAP1LC3) and, in consequence, promoted autophagic clearance of α-syn. Improved mitochondrial function and augmented autophagy caused by physical training were accompanied by ameliorated motor abilities in mice chronically treated with MPTP. Koo et al. (174) have also demonstrated that

training on the treadmill, applied after MPTP administration and lasting 8 weeks, reduced the loss of dopaminergic neurons, altered the level of proteins involved in apoptosis (increased Bcl-2 and decreased Bcl-2-associated X protein-Bax and caspase-3), increased expression of core proteins of the translocase of the outer mitochondrial membrane (TOM) complex, which is part of the mitochondrial import machinery (MIM), and increased the level of mitochondrial electron transport chain proteins (cyclooxygenase—COX-I, COX-IV, and mitochondrial 70 kDa heat shock protein—mtHSP70).

In the Al-Jarrah et al. (175) study, the authors have shown a positive impact of exercise training on the concentration of nitric oxide in the striatum of a chronic MPTP mouse model of PD. The 4-week training on the treadmill started after MPTP injections significantly decreased the level of neuronal nitric oxide synthase (nNOS) and inducible form of NOS (iNOS) in animals subjected to MPTP administration and treadmill training compared with the MPTP sedentary group. Four weeks of treadmill training improved the motor skills, evaluated through analyzing gait on the CatWalk, in a unilateral 6-OHDA rat model of PD (167, 168). This training also improved dopaminergic neuron viability, recovered mitochondrial function, and attenuated oxidative stress in PD rats. It was suggested that this phenomenon may be associated with improved mitochondrial turnover, that is, mitochondrial fusion, fission, and clearance, giving rise to increased quantities of mitochondria. It was demonstrated that exercise prevented 6-OHDA-induced loss of TH immunolabeling and activated the nuclear factor (erythroid-derived 2)-like 2 (Nrf2)–antioxidant response element (ARE) axis (i.e., ARE-dependent transcription) in the nigrostriatal pathway in C57BL/6 mice (176). The Nrf2-ARE is a major cellular defense mechanism against oxidative stress, which regulates the expression of antioxidant enzymes, such as gamma-glutamylcysteine ligase (γGCL) and HO-1. The Nrf2-ARE signaling pathway is strongly involved in neuroprotection and anti-inflammatory response. Treadmill training also stimulated mitochondrial biogenesis in the striatum of animals that were more resistant to the oxidant, 6-OHDA, and a nitric oxide donor, namely, (±)-*S*-nitroso-*N*-acetylpenicillamine. Similar results were obtained in the study of Tsou et al. (172) who showed that exercise enhanced the nigrostriatal Nrf2-mediated antioxidant defense capacity to protect dopaminergic neurons against MPP^+-induced toxicity in mice.

The study, designed to investigate the potential neuroprotective effect of swimming training in a mouse model of PD induced by 6-OHDA (24), demonstrated that a 4-week-long training attenuated the following features associated with PD: increased number of falls in the rotarod test, impairment of

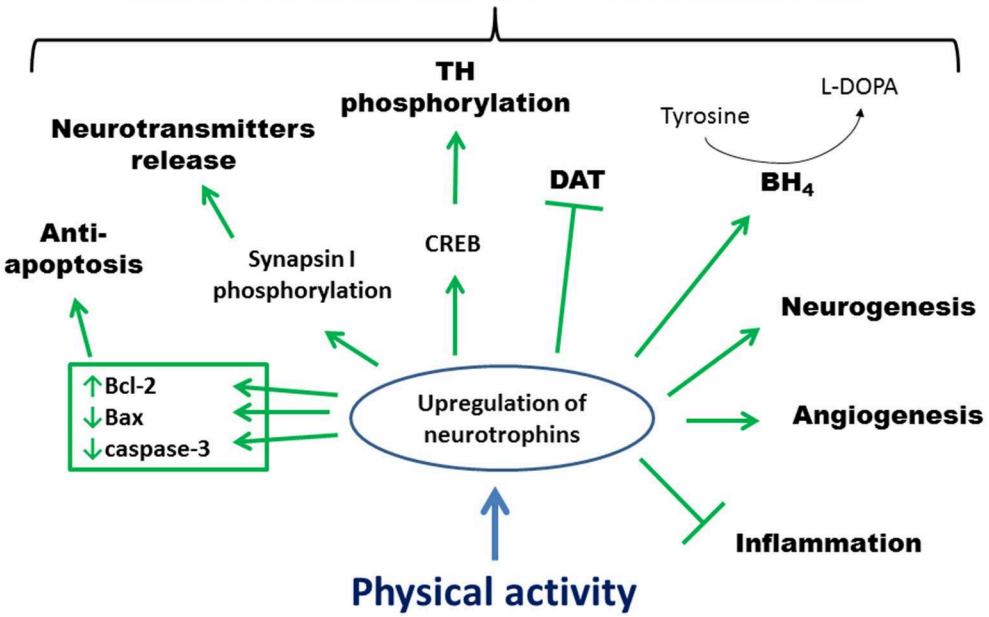

FIGURE 2 | Main processes involved in exercise-induced and neurotrophin-mediated increased viability of dopaminergic neurons. Physical activity leads to an increased level of neurotrophins in the brain. This upregulation stimulates anti-apoptotic proteins, mediates clustering and release of synaptic vesicles, activates CREB leading to phosphorylation and activation of TH, increases BH_4 level allowing conversion of tyrosine to L-DOPA, activates a pathway that inhibits DAT activity, increases blood flow and angiogenesis, enhances neurogenesis, and reduces inflammation. In summary, all these processes lead to greater survival of dopaminergic neurons and increased level of dopamine. Bcl-2, B-cell lymphoma 2; BH_4, tetrahydrobiopterin; CREB, cAMP response-element binding protein; DAT, dopamine transporter; L-DOPA, L-3,4-dihydroxyphenylalanine; TH, tyrosine hydroxylase.

long-term memory in the object recognition test, depressive-like behavior in the tail suspension test, and, at the same time, increase in ROS and IL-1β levels, inhibition of GPx activity, increase in GR and GST activities, and decrease in DA, HVA, and DOPAC levels.

Altogether, the findings presented in the above sections indicate that the beneficial effects induced by exercise, when neurodegenerative diseases, such as PD, are concerned, are mostly due to attenuation of oxidative stress and inflammation and elevation of NTFs.

CONCLUSIONS

Epidemiological data support the notion of beneficial effects of physical activity on preventing the risk, development, and progression of PD. Due to the fact that the effectiveness of pharmacological treatment decreases with the development of the disease and because properly selected physical training does not cause side effects, the physical activity should be recommended, as a supportive therapy, to patients suffering from PD. The physical activity not only reduces motor impairments but also improves cognitive functions.

Studies in animal models of PD indicate that physical activity may prevent the loss of, protect, or restore dopaminergic neurons probably by activating signaling cascades triggered by the increased availability of neurotrophins (**Figure 2**). Furthermore, neurotrophins can stabilize intracellular calcium concentration, induce antioxidant enzyme expression, and suppress the release of proinflammatory cytokines. Neurotrophic factors also provide important extracellular signals regulating neurogenesis in the adult brain. Finally, exercise improves motor circuitry through alterations in DA and glutamate neurotransmission.

However, animal models utilizing neurotoxins do not completely reproduce clinical symptoms and pathologies of PD in humans. Therefore, when MPTP is administered to animals, we are talking about inducing parkinsonism, not systemic PD. MPTP animal models are most often used to study the pathogenesis and progression of the disease and the effectiveness of drugs. Among the disadvantages of the animal model of PD caused by MPTP administration, the short-term and acute nature of pathological changes is mentioned. This disadvantage is remedied by the method of administration of neurotoxin used in chronic models of induction of parkinsonism in which the loss of dopaminergic neurons persists for at least several months. Another disadvantage is the difficulty or even impossibility to reproduce the characteristic movement disorders seen in humans. Lack of these disorders can be caused by a different organization of movement control by the nervous system in human and in mice. In animals, motor control is not so highly hierarchical and there is lesser contribution of the cerebral cortex and subcortical structures to locomotion than in humans.

It is likely that neurotoxin-based animal models of PD are adequate experimental models to study aspects of the beneficial effects of physical exertion, concerning changes at the tissue and cellular level and may be well-used to investigate such phenomena as neuroprotection, induction of trophic factors and neurogenesis, alleviation of inflammation, reduction of oxidative stress, and improvement of mitochondrial function. On the other hand, these models seems not so useful in studying the effect of exercise on alleviating movement disorders, because the results of the latest studies indicate that the loss of motor function after administration of neurotoxins is insignificant and difficult to capture/notice in the behavioral tests used.

It is possible that the outline, as presented in this review, of mechanisms by which physical activity confers neuroprotection is valid. However, more research is needed to confirm these mechanisms and elucidate their details. There is also a question what is the optimal type, intensity, and duration of the exercise when it should be performed to be maximally effective.

Specifically, as the majority of data supporting the ability of physical activity to protect DA neurons stems from neurotoxin PD models, the question arises whether this property of physical activity would persist in other animal models of PD. With a positive answer to this question, the next issue to be cleared would be whether there are common neuroprotective mechanisms. The existence of such common mechanisms will be strongly supportive to continue physical activity by PD patients during the whole course of illness as a disease-modifying factor.

AUTHOR CONTRIBUTIONS

EP, WN, and GN contributed equally to this work and wrote the main body of the paper. AG wrote the Introduction section. AW and AS made the figures and create the references. All authors reviewed the final manuscript.

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REFERENCES

1. Muhlack S, Welnic J, Woitalla D, Müller T. Exercise improves efficacy of levodopa in patients with Parkinson's disease. *Mov Disord.* (2007) 22:427–30. doi: 10.1002/mds.21346
2. Fayyaz M, Jaffery SS, Anwer F, Zil-E-Ali A, Anjum I. The effect of physical activity in Parkinson's disease: a mini-review. *Cureus.* (2018) 10:e2995. doi: 10.7759/cureus.2995
3. Petzinger G, Fisher B, McEwen S, Beeler S, Walsh J, Jakowec MENT. Exercise-enhanced neuroplasticity targeting motor and cognitive circuitry in Parkinson's disease. *Lancet Neurol.* (2013) 12:716–26. doi: 10.1016/S1474-4422(13)70123-6
4. Reuter I, Mehnert S, Leone P, Kaps M, Oechsner M, Engelhardt M. Effects of a flexibility and relaxation programme, walking, and nordic walking on Parkinson's disease. *J Aging Res.* (2011) 2011:1–18. doi: 10.4061/2011/232473

5. Szefler-Derela J, Suszynski K, Doroniewicz I, Kowalczyk A, Opala G, Kwiek SJ. Nordic walking in Parkinson disease rehabilitation. *Ann Acad Medicae Silesiensis*. (2014) 68: 361–7.
6. Ebersbach G, Ebersbach A, Gandor F, Wegner B, Wissel J, Kupsch A. Impact of physical exercise on reaction time in patients with Parkinson's disease—data from the Berlin BIG study. *Arch Phys Med Rehabil*. (2014) 95:996–9. doi: 10.1016/j.apmr.2013.10.020
7. Cugusi L, Solla P, Serpe R, Carzedda T, Piras L, Oggianu M, et al. Effects of a nordic walking program on motor and non-motor symptoms, functional performance and body composition in patients with Parkinson's disease. *NeuroRehabilitation*. (2015) 37:245–54. doi: 10.3233/NRE-151257
8. Monteiro EP, Franzoni LT, Cubillos DM, de Oliveira Fagundes A, Carvalho AR, Oliveira HB, et al. Effects of Nordic walking training on functional parameters in Parkinson's disease: a randomized controlled clinical trial. *Scand J Med Sci Sports*. (2017) 27:351–8. doi: 10.1111/sms.12652
9. Bello O, Sanchez JA, Lopez-Alonso V, Márquez G, Morenilla L, Castro X, et al. The effects of treadmill or overground walking training program on gait in Parkinson's disease. *Gait Posture*. (2013) 38:590–5. doi: 10.1016/j.gaitpost.2013.02.005
10. Nadeau A, Pourcher E, Corbeil P. Effects of 24 wk of treadmill training on gait performance in Parkinson's disease. *Med Sci Sport Exerc*. (2014) 46:645–55. doi: 10.1249/MSS.0000000000000144
11. Scandalis TA, Bosak A, Berliner JC, Helman LL, Wells MR. Resistance training and gait function in patients with Parkinson's disease. *Am J Phys Med Rehabil*. (2001) 80:38–43. doi: 10.1097/00002060-200101000-00011
12. Hirsch MA, Toole T, Maitland CG, Rider RA. The effects of balance training and high-intensity resistance training on persons with idiopathic Parkinson's disease. *Arch Phys Med Rehabil*. (2003) 84:1109–17. doi: 10.1016/S0003-9993(03)00046-7
13. Cruise KE, Bucks RS, Loftus AM, Newton RU, Pegoraro R, Thomas MG. Exercise and Parkinson's: benefits for cognition and quality of life. *Acta Neurol Scand*. (2011) 123:13–9. doi: 10.1111/j.1600-0404.2010.01338.x
14. Tabak R, Aquije G, Fisher BE. Aerobic exercise to improve executive function in Parkinson disease. *J Neurol Phys Ther*. (2013) 37:58–64. doi: 10.1097/NPT.0b013e31829219bc
15. Nocera JR, Altmann LJP, Sapienza C, Okun MS, Hass CJ. Can exercise improve language and cognition in Parkinson's disease? A case report. *Neurocase*. (2010) 16:301–6. doi: 10.1080/13554790903559663
16. LaHue SC, Comella CL, Tanner CM. The best medicine? The influence of physical activity and inactivity on Parkinson's disease. *Mov Disord*. (2016) 31:1444–54. doi: 10.1002/mds.26728
17. Phillips C, Baktir MA, Srivatsan M, Salehi A. Neuroprotective effects of physical activity on the brain: a closer look at trophic factor signaling. *Front Cell Neurosci*. (2014) 8:170. doi: 10.3389/fncel.2014.00170
18. Sleiman SF, Henry J, Al-Haddad R, El Hayek L, Abou Haidar E, Stringer T, et al. Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body β-hydroxybutyrate. *Elife*. (2016) 5:e15092 doi: 10.7554/eLife.15092
19. Lau Y-S, Patki G, Das-Panja K, Le W-D, Ahmad SO. Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. *Eur J Neurosci*. (2011) 33:1264–74. doi: 10.1111/j.1460-9568.2011.07626.x
20. Gerecke KM, Jiao Y, Pagala V, Smeyne RJ. Exercise does not protect against MPTP-induced neurotoxicity in BDNF happloinsufficent mice. *PLoS ONE*. (2012) 7:1–11. doi: 10.1371/journal.pone.0043250
21. Sconce MD, Churchill MJ, Greene RE, Meshul CK. Intervention with exercise restores motor deficits but not nigrostriatal loss in a progressive MPTP mouse model of Parkinson's disease. *Neuroscience*. (2015) 299:156–74. doi: 10.1016/j.neuroscience.2015.04.069
22. Kintz N, Petzinger GM, Akopian G, Ptasnik S, Williams C, Jakowec MW, et al. Exercise modifies α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor expression in striatopallidal neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse. *J Neurosci Res*. (2013) 91:1492–507. doi: 10.1002/jnr.23260
23. Chalimoniuk M, Jagsz S, Sadowska-Krepa E, Chrapusta SJ, Klapcinska B, Langfort J. Diversity of endurance training effects on antioxidant defenses and oxidative damage in different brain regions of adolescent male rats. *J Physiol Pharmacol*. (2015) 66:539–47.
24. Goes ATR, Souza LC, Filho CB, Del Fabbro L, De Gomes MG, Boeira SP, et al. Neuroprotective effects of swimming training in a mouse model of Parkinson's disease induced by 6-hydroxydopamine. *Neuroscience*. (2014) 256:61–71. doi: 10.1016/j.neuroscience.2013.09.042
25. Real CC, Garcia PC, Britto LRG. Treadmill exercise prevents increase of neuroinflammation markers involved in the dopaminergic damage of the 6-OHDA Parkinson's disease model. *J Mol Neurosci*. (2017) 63:36–49. doi: 10.1007/s12031-017-0955-4
26. Ambrogini P, Lattanzi D, Ciuffoli S, Betti M, Fanelli M, Cuppini R. Physical exercise and environment exploration affect synaptogenesis in adult-generated neurons in the rat dentate gyrus: possible role of BDNF. *Brain Res*. (2013) 1534:1–12. doi: 10.1016/j.brainres.2013.08.023
27. Lopez-Lopez C, LeRoith D, Torres-Aleman I. Insulin-like growth factor I is required for vessel remodeling in the adult brain. *Proc Natl Acad Sci USA*. (2004) 101:9833–8. doi: 10.1073/pnas.0400337101
28. Van der Borght K, Kóbor-Nyakas DÉ, Klauke K, Eggen BJL, Nyakas C, Van der Zee EA, et al. Physical exercise leads to rapid adaptations in hippocampal vasculature: Temporal dynamics and relationship to cell proliferation and neurogenesis. *Hippocampus*. (2009) 19:928–36. doi: 10.1002/hipo.20545
29. van Praag H, Shubert T, Zhao C, FH G. Exercise enhances learning and hippocampal neurogenesis in aged mice. *J Neurosci*. (2005) 25:8680–5. doi: 10.1523/JNEUROSCI.1731-05.2005
30. Fredriksson A, Archer T. MPTP-induced behavioural and biochemical deficits: a parametric analysis. *J Neural Transm Park Dis Dement Sect*. (1994) 7:123–32. doi: 10.1007/BF02260967
31. Rozas G, López-Martín E, Guerra MJ, Labandeira-García JL. The overall rod performance test in the MPTP-treated-mouse model of Parkinsonism. *J Neurosci Methods*. (1998) 83:165–75. doi: 10.1016/S0165-0270(98)00078-8
32. Tillerson JL, Caudle WM, Reverón ME, Miller GW. Detection of behavioral impairments correlated to neurochemical deficits in mice treated with moderate doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Exp Neurol*. (2002) 178:80–90. doi: 10.1006/exnr.2002.8021
33. Chung YC, Baek JY, Kim SR, Ko HW, Bok E, Shin W-H, et al. Capsaicin prevents degeneration of dopamine neurons by inhibiting glial activation and oxidative stress in the MPTP model of Parkinson's disease. *Exp Mol Med*. (2017) 49:e298. doi: 10.1038/emm.2016.159
34. Wu SY, Wang TF, Yu L, Jen CJ, Chuang JI, Wu FS, et al. Running exercise protects the substantia nigra dopaminergic neurons against inflammation-induced degeneration via the activation of BDNF signaling pathway. *Brain Behav Immun*. (2011) 25:135–46. doi: 10.1016/j.bbi.2010.09.006
35. Hsueh SC, Chen KY, Lai JH, Wu CC, Yu YW, Luo Y, et al. Voluntary physical exercise improves subsequent motor and cognitive impairments in a rat model of Parkinson's disease. *Int J Mol Sci*. (2018) 19:508. doi: 10.3390/ijms19020508
36. Rafie F, Shabazi M, Sheikh M, Naghdi N, Sheibani V. Effects of voluntary exercise on motor function in Parkinson's disease model of rats. *Ann Appl Sport Sci*. (2017) 5:81–6. doi: 10.18869/acadpub.aassjournal.5.2.81
37. Heikkila RE, Sonsalla PK. The MPTP-treated mouse as a model of Parkinsonism: how good is it? *Neurochem Int*. (1992) 20:299–303. doi: 10.1016/0197-0186(92)90256-Q
38. Sundström E, Fredriksson A, Archer T. Chronic neurochemical and behavioral changes in MPTP-lesioned C57BL/6 mice: a model for Parkinson's disease. *Brain*. (1990) 28:181–8. doi: 10.1016/0006-8993(90)91656-2
39. Bové J, Perier C. Neurotoxin-based models of Parkinson's disease. *Neuroscience*. (2012) 211:51–76. doi: 10.1016/j.neuroscience.2011.10.057
40. Blandini F, Armentero M-T. Animal models of Parkinson's disease. *FEBS J*. (2012) 279:1156–66. doi: 10.1111/j.1742-4658.2012.08491.x
41. Bazzu G, Calia G, Puggioni G, Spissu Y, Rocchitta G, Debetto P, et al. alpha-Synuclein- and MPTP-generated rodent models of Parkinson's disease and the study of extracellular striatal dopamine dynamics: a microdialysis approach. *CNS Neurol Disord Drug Targets*. (2010) 9:482–90. doi: 10.2174/187152710791556177
42. Pothakos K, Kurz MJ, Lau Y-S. Restorative effect of endurance exercise on behavioral deficits in the chronic mouse model of Parkinson's disease with severe neurodegeneration. *BMC Neurosci*. (2009) 10:6. doi: 10.1186/1471-2202-10-6

43. Tuon T, Valvassori SS, Lopes-Borges J, Luciano T, Trom CB, Silva LA, et al. Physical training exerts neuroprotective effects in the regulation of neurochemical factors in an animal model of Parkinson's disease. *Neuroscience*. (2012) 227:305–12. doi: 10.1016/j.neuroscience.2012.09.063
44. Fisher BE, Petzinger GM, Nixon K, Hogg E, Bremmer S, Meshul CK, et al. Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia. *J Neurosci Res*. (2004) 77:378–90. doi: 10.1002/jnr.20162
45. Jang Y, Kwon I, Song W, Cosio-Lima LM, Lee Y. Endurance exercise mediates neuroprotection against MPTP-mediated Parkinson's disease via enhanced neurogenesis, antioxidant capacity, and autophagy. *Neuroscience*. (2018) 379:292–301. doi: 10.1016/j.neuroscience.2018.03.015
46. Chen YH, Kuo TT, Kao JH, Huang EYK, Hsieh TH, Chou YC, et al. Exercise ameliorates motor deficits and improves dopaminergic functions in the rat hemi-Parkinson's model. *Sci Rep*. (2018) 8:3973. doi: 10.1038/s41598-018-22462-y
47. Chen Y, Zhang Y, Li L, Hölscher C. Neuroprotective effects of geniposide in the MPTP mouse model of Parkinson's disease. *Eur J Pharmacol*. (2015) 768:21–7. doi: 10.1016/j.ejphar.2015.09.029
48. Lu M, Su C, Qiao C, Bian Y, Ding J, Hu G. Metformin prevents dopaminergic neuron death in MPTP/P-induced mouse model of Parkinson's disease via autophagy and mitochondrial ROS clearance. *Int J Neuropsychopharmacol*. (2016) 19:pyw047. doi: 10.1093/ijnp/pyw047
49. Liu J, Huang D, Xu J, Tong J, Wang Z, Huang L, et al. Tiagabine protects dopaminergic neurons against neurotoxins by inhibiting microglial activation. *Sci Rep*. (2015) 5:15720. doi: 10.1038/srep15720
50. Klein C, Rasinska J, Empl L, Sparenberg M, Poshtiban A, Hain EG, et al. Physical exercise counteracts MPTP-induced changes in neural precursor cell proliferation in the hippocampus and restores spatial learning but not memory performance in the water maze. *Behav Brain Res*. (2016) 307:227–38. doi: 10.1016/j.bbr.2016.02.040
51. Sung Y-H, Kim S-C, Hong H-P, Park C-Y, Shin M-S, Kim C-J, et al. Treadmill exercise ameliorates dopaminergic neuronal loss through suppressing microglial activation in Parkinson's disease mice. *Life Sci*. (2012) 91:1309–16. doi: 10.1016/j.lfs.2012.10.003
52. Lau YS, Fung YK, Trobough KL, Cashman JR, Wilson JA. Depletion of striatal dopamine by the N-oxide of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Neurotoxicology*. (1991) 12:189–99.
53. Meredith GE, Rademacher DJ. MPTP mouse models of Parkinson's disease: an update. *J Park Dis*. (2012) 1:19–33. doi: 10.3233/JPD-2011-11023
54. Zhang Q, Heng Y, Mou Z, Huang J, Yuan Y, Chen N. Reassessment of subacute MPTP-treated mice as animal model of Parkinson's disease. *Acta Pharmacol Sin*. (2017) 38:1317–28. doi: 10.1038/aps.2017.49
55. Schamne MG, Mack JM, Moretti M, Matheus FC, Walz R, Lanfumey L, et al. The gender-biased effects of intranasal MPTP administration on anhedonic- and depressive-like behaviors in C57BL/6 mice: the role of neurotrophic factors. *Neurotox Res*. (2018) 34:808–19. doi: 10.1007/s12640-018-9912-4
56. Tillerson JL, Caudle WM, Reverón ME, Miller GW. Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease. *Neuroscience*. (2003) 119:899–911. doi: 10.1016/S0306-4522(03)00096-4
57. Fleming SM. Early and progressive sensorimotor anomalies in mice overexpressing wild-type human -synuclein. *J Neurosci*. (2004) 24:9434–40. doi: 10.1523/JNEUROSCI.3080-04.2004
58. Caudle WM, Richardson JR, Wang MZ, Taylor TN, Guillot TS, McCormack AL, et al. Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *J Neurosci*. (2007) 27:8138–48. doi: 10.1523/JNEUROSCI.0319-07.2007
59. Manning-Bo AB, Caudle WM, Perez XA, Reaney SH, Paletzki R, Isla MZ, et al. Increased vulnerability of nigrostriatal terminals in DJ-1-deficient mice is mediated by the dopamine transporter. *Neurobiol Dis*. (2007) 27:141–50. doi: 10.1016/j.nbd.2007.03.014
60. Price DA, Martinez AA, Seillier A, Koek W, Acosta Y, Fernandez E, et al. WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Eur J Neurosci*. (2009) 29:2177–86. doi: 10.1111/j.1460-9568.2009.06764.x
61. Kim ST, Son HJ, Choi JH, Ji IJ, Hwang O. Vertical grid test and modified horizontal grid test are sensitive methods for evaluating motor dysfunctions in the MPTP mouse model of Parkinson's disease. *Brain Res*. (2010) 1306:176–83. doi: 10.1016/j.brainres.2009.09.103
62. Salama M, ElDakroory SAEA, ElTantawy D, Ghanem AA, Elghaffar HA, Elhusseiny M, ElHak SG. Regenerative effects of umbilical cord matrix cells (UCMCs) in a rodent model of rotenone neurotoxicity. *Environ Toxicol Pharmacol*. (2012) 34:338–44. doi: 10.1016/j.etap.2012.05.005
63. Korecka JA, Eggers R, Swaab DF, Bossers K, Verhaagen J. Modeling early Parkinson's disease pathology with chronic low dose MPTP treatment. *Restor Neurol Neurosci*. (2013) 31:155–67. doi: 10.3233/RNN-110222
64. Niewiadomski W, Palasz E, Skupinska M, Zylinski M, Steczkowska M, Gasiorowska A, et al. TracMouse: a computer aided movement analysis script for the mouse inverted horizontal grid test. *Sci Rep*. (2016) 6:39331. doi: 10.1038/srep39331
65. Gerecke KM, Jiao Y, Pani A, Pagala V, Smeyne RJ. Exercise protects against MPTP-induced neurotoxicity in mice. *Brain Res*. (2010) 1341:72–83. doi: 10.1016/j.brainres.2010.01.053
66. Ahmad SO, Park JH, Stenho-Bittel L, Lau YS. Effects of endurance exercise on ventral tegmental area neurons in the chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid-treated mice. *Neurosci Lett*. (2009) 450:102–5. doi: 10.1016/j.neulet.2008.11.065
67. Al-Jarrah M, Pothakos K, Novikova L, Smirnova IV, Kurz MJ, Stehno-Bittel L, et al. Endurance exercise promotes cardiorespiratory rehabilitation without neurorestoration in the chronic mouse model of Parkinsonism with severe neurodegeneration. *Neuroscience*. (2007) 149:28–37. doi: 10.1016/j.neuroscience.2007.07.038
68. Zhao L, He LX, Huang SN, Gong LJ, Li L, Lv YY, et al. Protection of dopamine neurons by vibration training and up-regulation of brain-derived neurotrophic factor in a MPTP mouse model of Parkinson's disease. *Physiol Res*. (2014) 63:649–57.
69. Koo J-H, Cho J-Y. Treadmill exercise attenuates α-synuclein levels by promoting mitochondrial function and autophagy possibly via SIRT1 in the chronic MPTP/P-induced mouse model of Parkinson's disease. *Neurotox Res*. (2017) 32:473–86. doi: 10.1007/s12640-017-9770-5
70. Tajiri N, Yasuhara T, Shingo T, Kondo A, Yuan W, Kadota T, et al. Exercise exerts neuroprotective effects on Parkinson's disease model of rats. *Brain Res*. (2010) 1310:200–7. doi: 10.1016/j.brainres.2009.10.075
71. Palasz E, Niewiadomski W, Gasiorowska A, Mietelska-Porowska A, Niewiadomska G. Neuroplasticity and neuroprotective effect of treadmill training in the chronic mouse model of Parkinson's disease. *Neural Plast*. (2019) 2019:1–14. doi: 10.1155/2019/8215017
72. Shin M-S, Jeong H-Y, An D-I, Lee H-Y, Sung Y-H. Treadmill exercise facilitates synaptic plasticity on dopaminergic neurons and fibers in the mouse model with Parkinson's disease. *Neurosci Lett*. (2016) 621:28–33. doi: 10.1016/j.neulet.2016.04.015
73. Zhang L, Feng Y, Ji W, Liu J, Liu K. Effect of voluntary wheel running on striatal dopamine level and neurocognitive behaviors after molar loss in rats. *Behav Neurol*. (2017) 2017:1–6. doi: 10.1155/2017/6137071
74. Brichta L, Greengard P. Molecular determinants of selective dopaminergic vulnerability in Parkinson's disease: an update. *Front Neuroanat*. (2014) 8:152. doi: 10.3389/fnana.2014.00152
75. Olanow CW, Agid Y, Mizuno Y, Albanese A, Bonucelli U, Damier P, et al. Levodopa in the treatment of Parkinson's disease: current controversies. *Mov Disord*. (2004) 19:997–1005. doi: 10.1002/mds.20243
76. Burke RE, O'Malley K. Axon degeneration in Parkinson's disease. *Exp Neurol*. (2013) 246:72–83. doi: 10.1016/j.expneurol.2012.01.011
77. Toy WA, Petzinger GM, Leyshon BJ, Akopian GK, Walsh JP, Hoffman MV, et al. Treadmill exercise reverses dendritic spine loss in direct and indirect striatal medium spiny neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *Neurobiol Dis*. (2014) 63:201–9. doi: 10.1016/j.nbd.2013.11.017
78. Pisani A, Centonze D, Bernardi G, Calabresi P. Striatal synaptic plasticity: implications for motor learning and Parkinson's disease. *Mov Disord*. (2005) 20:395–402. doi: 10.1002/mds.20394
79. Yager LM, Garcia AF, Wunsch AM, Ferguson SM. The ins and outs of the striatum: role in drug addiction. *Neuroscience*. (2015) 301:529–41. doi: 10.1016/j.neuroscience.2015.06.033

80. Petzinger GM, Holschneider DP, Fisher BE, McEwen S, Kintz N, Halliday M, et al. The effects of exercise on dopamine neurotransmission in Parkinson's disease: targeting neuroplasticity to modulate basal ganglia circuitry. *Brain Plast*. (2015) 1:29–39. doi: 10.3233/BPL-150021
81. Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary? *Eur J Neurosci*. (2004) 19:2455–63. doi: 10.1111/j.0953-816X.2004.03344.x
82. Chauhan NB, Siegel GJ, Lee JM. Depletion of glial cell line-derived neurotrophic factor in substantia nigra neurons of Parkinson's disease brain. *J Chem Neuroanat*. (2001) 21:277–88. doi: 10.1016/S0891-0618(01)00115-6
83. Siegel GJ, Chauhan NB, Service N, Hines E, Hospital VA, Rm F. Neurotrophic factors in Alzheimer's and Parkinson's disease brain. *Brain Res Rev*. (2000) 33:199–227. doi: 10.1016/S0165-0173(00)00030-8
84. Parain K, Murer M, Yan Q, Faucheux B, Agid Y, Hirsch E, et al. Reduced expression of brain derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport*. (1999) 10:557–61. doi: 10.1097/00001756-199902250-00021
85. Sharma S, Deshmukh R. Vinpocetine attenuates MPTP-induced motor deficit and biochemical abnormalities in Wistar rats. *Neuroscience*. (2015) 286:393–403. doi: 10.1016/j.neuroscience.2014.12.008
86. Schwenkgrub J, Zaremba M, Joniec-Maciejak I, Cudna A, Mirowska-Guzel D, Kurkowska-Jastrzebska I. The phosphodiesterase inhibitor, ibudilast, attenuates neuroinflammation in the MPTP model of Parkinson's disease. *PLoS ONE*. (2017) 12:e0182019. doi: 10.1371/journal.pone.0182019
87. Sharma S, Kumar K, Deshmukh R, Sharma PL. Phosphodiesterases: regulators of cyclic nucleotide signals and novel molecular target for movement disorders. *Eur J Pharmacol*. (2013) 714:486–97. doi: 10.1016/j.ejphar.2013.06.038
88. Aron L, Klein R. Repairing the parkinsonian brain with neurotrophic factors. *Trends Neurosci*. (2011) 34:88–100. doi: 10.1016/j.tins.2010.11.001
89. Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science*. (1993) 260:1130–2. doi: 10.1126/science.8493557
90. Hyman C, Hofer M, Barde Y-A, Juhasz M, Yancopoulos GD, Squinto SP, et al. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature*. (1991) 350:230–2. doi: 10.1038/350230a0
91. Tome D, Fonseca C, Campos F, Baltazar G. Role of neurotrophic factors in Parkinson's disease. *Curr Pharm Des*. (2017) 23:809–38. doi: 10.2174/1381612822666161208120422
92. Mohammadi A, Amooeian VG, Rashidi E. Dysfunction in brain-derived neurotrophic factor signaling pathway and susceptibility to Schizophrenia, Parkinson's and Alzheimer's diseases. *Curr Gene Ther*. (2018) 18:45–63. doi: 10.2174/1566523218666180302163029
93. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci*. (2015) 6:1164–78. doi: 10.5114/aoms.2015.56342
94. Kramer ER, Liss B. GDNF-Ret signaling in midbrain dopaminergic neurons and its implication for Parkinson disease. *FEBS Lett*. (2015) 589:3760–72. doi: 10.1016/j.febslet.2015.11.006
95. Tenenbaum L, Humbert-Claude M. Glial cell line-derived neurotrophic factor gene delivery in Parkinson's disease: a delicate balance between neuroprotection, trophic effects, and unwanted compensatory mechanisms. *Front Neuroanat*. (2017) 11:29. doi: 10.3389/fnana.2017.00029
96. Lee E-R, Kim J-Y, Kang Y-J, Ahn J-Y, Kim J-H, Kim B-W, et al. Interplay between PI3K/Akt and MAPK signaling pathways in DNA-damaging drug-induced apoptosis. *Biochim Biophys Acta*. (2006) 1763:958–68. doi: 10.1016/j.bbamcr.2006.06.006
97. García-Martínez JM, Pérez-Navarro E, Gavaldà N, Alberch J. Glial cell line-derived neurotrophic factor promotes the arborization of cultured striatal neurons through the p42/p44 mitogen-activated protein kinase pathway. *J Neurosci Res*. (2006) 83:68–79. doi: 10.1002/jnr.20713
98. Lindgren N, Leak RK, Carlson KM, Smith AD, Zigmond MJ. Activation of the extracellular signal-regulated kinases 1 and 2 by glial cell line-derived neurotrophic factor and its relation to neuroprotection in a mouse model of Parkinson's disease. *J Neurosci Res*. (2008) 86:2039–49. doi: 10.1002/jnr.21641
99. Zhu G, Li J, He L, Wang X, Hong X. MPTP-induced changes in hippocampal synaptic plasticity and memory are prevented by memantine through the BDNF-TrkB pathway. *Br J Pharmacol*. (2015) 172:2354–68. doi: 10.1111/bph.13061
100. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci USA*. (1999) 96:13427–31. doi: 10.1073/pnas.96.23.13427
101. Yasuhara T, Hara K, Maki M, Matsukawa N, Fujino H, Date I, et al. Lack of exercise, via hindlimb suspension, impedes endogenous neurogenesis. *Neuroscience*. (2007) 149:182–91. doi: 10.1016/j.neuroscience.2007.07.045
102. Yi SS, Hwang IK, Yoo K-Y, Park OK, Yu J, Yan B, et al. Effects of treadmill exercise on cell proliferation and differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. *Neurochem Res*. (2009) 34:1039–46. doi: 10.1007/s11064-008-9870-y
103. Wu C-W, Chen Y-C, Yu L, Chen H, Jen CJ, Huang A-M, et al. Treadmill exercise counteracts the suppressive effects of peripheral lipopolysaccharide on hippocampal neurogenesis and learning and memory. *J Neurochem*. (2007) 103:2471–81. doi: 10.1111/j.1471-4159.2007.04987.x
104. Smith AD, Zigmond MJ. Can the brain be protected through exercise? Lessons from an animal model of parkinsonism. *Exp Neurol*. (2003) 184:31–9. doi: 10.1016/j.expneurol.2003.08.017
105. Teismann P, Schulz JB. Cellular pathology of Parkinson's disease: astrocytes, microglia and inflammation. *Cell Tissue Res*. (2004) 318:149–61. doi: 10.1007/s00441-004-0944-0
106. McGeer PL, Kawamata T, Walker DG, Akiyama H, Tooyama I, McGeer EG. Microglia in degenerative neurological disease. *Glia*. (1993) 7:84–92. doi: 10.1002/glia.440070114
107. Dehmer T, Lindenau J, Haid S, Dichgans J, Schulz JB. Deficiency of inducible nitric oxide synthase protects against MPTP toxicity in vivo. *J Neurochem*. (2000) 74:2213–6. doi: 10.1046/j.1471-4159.2000.0742213.x
108. Kohutnicka M, Lewandowska E, Kurkowska-Jastrzebska I, Członkowski A, Członkowska A. Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Immunopharmacology*. (1998) 39:167–80. doi: 10.1016/S0162-3109(98)00022-8
109. Lofrumento DD, Saponaro C, Cianciulli A, De Nuccio F, Mitolo V, Nicolardi G, et al. MPTP-induced neuroinflammation increases the expression of pro-inflammatory cytokines and their receptors in mouse brain. *Neuroimmunomodulation*. (2011) 18:79–88. doi: 10.1159/000320027
110. Kurkowska-Jastrzebska I, Wronska A, Kohutnicka M, Członkowski A, Członkowska A. The inflammatory reaction following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxication in mouse. *Exp Neurol*. (1999) 156:50–61. doi: 10.1006/exnr.1998.6993
111. Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest*. (2008) 119:182–92. doi: 10.1172/JCI36470
112. Baquet ZC. Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. *J Neurosci*. (2005) 25:6251–9. doi: 10.1523/JNEUROSCI.4601-04.2005
113. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol Ser A*. (2014) 69:S4–9. doi: 10.1093/gerona/glu057
114. Benarroch EE. Neuron-astrocyte interactions: partnership for normal function and disease in the central nervous system. *Mayo Clin Proc*. (2005) 80:1326–38. doi: 10.4065/80.10.1326
115. Pita-Almenar JD, Zou S, Colbert CM, Eskin A. Relationship between increase in astrocytic GLT-1 glutamate transport and late-LTP. *Learn Mem*. (2012) 19:615–26. doi: 10.1101/lm.023259.111
116. Walker KA. Inflammation and neurodegeneration: chronicity matters. *Aging*. (2018) 11:3–4. doi: 10.18632/aging.101704
117. McGeer PL. Inflammation and the degenerative diseases of aging. *Ann N Y Acad Sci USA*. (2004) 1035:104–16. doi: 10.1196/annals.1332.007
118. Vivekanantham S, Shah S, Dewji R, Dewji A, Khatri C, Ologunde R. Neuroinflammation in Parkinson's disease: role in neurodegeneration and tissue repair. *Int J Neurosci*. (2015) 125:717–25. doi: 10.3109/00207454.2014.982795

119. Carta AR, Mulas G, Bortolanza M, Duarte T, Pillai E, Fisone G, et al. l-DOPA-induced dyskinesia and neuroinflammation: do microglia and astrocytes play a role? *Eur J Neurosci*. (2017) 45:73–91. doi: 10.1111/ejn.13482
120. Haggerty T, Credle J, Rodriguez O, Wills J, Oaks AW, Masliah E, et al. Hyperphosphorylated Tau in an α-synuclein-overexpressing transgenic model of Parkinson's disease. *Eur J Neurosci*. (2011) 33:1598–610. doi: 10.1111/j.1460-9568.2011.07660.x
121. Rodrigues RW, Gomide VC, Chadi G. Astroglial and microglial reaction after a partial nigrostriatal degeneration induced by the striatal injection of different doses of 6-hydroxydopamine. *Int J Neurosci*. (2001) 109:91–126. doi: 10.3109/00207450108986528
122. Liu J, Wang MW, Gu P, Ma QY, Wang YY, Geng Y, et al. Microglial activation and age-related dopaminergic neurodegeneration in MPTP-treated SAMP8 mice. *Brain Res*. (2010) 1345:213–20. doi: 10.1016/j.brainres.2010.05.043
123. Sawada H, Hishida R, Hirata Y, Ono K, Suzuki H, Muramatsu S, et al. Activated microglia affect the nigro-striatal dopamine neurons differently in neonatal and aged mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J Neurosci Res*. (2007) 85:1752–61. doi: 10.1002/jnr.21241
124. Bian M juan, Li L mei, Yu M, Fei J, Huang F. Elevated interleukin-1β induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine aggravating dopaminergic neurodegeneration in old male mice. *Brain Res*. (2009) 1302:256–64. doi: 10.1016/j.brainres.2009.07.030
125. Wang XJ, Zhang S, Yan ZQ, Zhao YX, Zhou HY, Wang Y, et al. Impaired CD200-CD200R-mediated microglia silencing enhances midbrain dopaminergic neurodegeneration: Roles of aging, superoxide, NADPH oxidase, and p38 MAPK. *Free Radic Biol Med*. (2011) 50:1094–106. doi: 10.1016/j.freeradbiomed.2011.01.032
126. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactinve astroctes are induced by activated microglia. *Nature*. (2017) 541:481–7. doi: 10.1038/nature21029
127. Neal M, Richardson JR. Epigenetic regulation of astrocyte function in neuroinflammation and neurodegeneration. *Biochim Biophys Acta*. (2018) 1864:432–43. doi: 10.1016/j.bbadis.2017.11.004
128. Al-Jarrah MD, Jamous M. Effect of endurance exercise training on the expression of GFAP, S100B, and NSE in the striatum of chronic/progressive mouse model of Parkinson's disease. *NeuroRehabilitation*. (2011) 28:359–63. doi: 10.3233/NRE-2011-0664
129. Cotman CW, Berchtold NC, Christie L-A. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci*. (2007) 30:464–72. doi: 10.1016/j.tins.2007.06.011
130. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol*. (2011) 11:607–10. doi: 10.1038/nri3041
131. Dimatelis JJ, Hendricks S, Hsieh J, Vlok NM, Bugarith K, Daniels WMU, et al. Exercise partly reverses the effect of maternal separation on hippocampal proteins in 6-hydroxydopamine-lesioned rat brain. *Exp Physiol*. (2013) 98:233–44. doi: 10.1113/expphysiol.2012.066720
132. Jang Y, Koo JH, Kwon I, Kang EB, Um HS, Soya H, et al. Neuroprotective effects of endurance exercise against neuroinflammation in MPTP-induced Parkinson's disease mice. *Brain Res*. (2017) 1655:186–93. doi: 10.1016/j.brainres.2016.10.029
133. Łabuzek K, Skrudlik E, Gabryel B, Okopien B. Anti-inflammatory microglial cell function in the light of the latest scientific research. *Ann Acad Medicae Silesiensis*. (2015) 69:99–110. doi: 10.18794/aams/32608
134. McGeer PL, McGeer EG. Glial reactions in Parkinson's disease. *Mov Disord*. (2008) 23:474–83. doi: 10.1002/mds.21751
135. Saavedra A, Baltazar G, Duarte EP. Interleukin-1β mediates GDNF up-regulation upon dopaminergic injury in ventral midbrain cell cultures. *Neurobiol Dis*. (2007) 25:92–104. doi: 10.1016/j.nbd.2006.08.019
136. Cobianchi S, Arbat-Plana A, Lopez-Alvarez VM, Navarro X. Neuroprotective effects of exercise treatments after injury: the dual role of neurotrophic factors. *Curr Neuropharmacol*. (2017) 15:495–518. doi: 10.2174/1570159X14666160330105132
137. Yuskaitis CJ, Jope RS. Glycogen synthase kinase-3 regulates microglial migration, inflammation, and inflammation-induced neurotoxicity. *Cell Signal*. (2009) 21:264–73. doi: 10.1016/j.cellsig.2008.10.014
138. Maixner DW, Weng H-R. The role of glycogen synthase kinase 3 beta in neuroinflammation and pain. *J Pharm Pharmacol*. (2013) 1:001. doi: 10.13188/2327-204X.1000001
139. Wang M-J, Huang H-Y, Chen W-F, Chang H-F, Kuo J-S. Glycogen synthase kinase-3β inactivation inhibits tumor necrosis factor-α production in microglia by modulating nuclear factor κB and MLK3/JNK signaling cascades. *J Neuroinflammation*. (2010) 7:99. doi: 10.1186/1742-2094-7-99
140. Green HF, Nolan YM. GSK-3 mediates the release of IL-1β, TNF-α and IL-10 from cortical glia. *Neurochem Int*. (2012) 61:666–71. doi: 10.1016/j.neuint.2012.07.003
141. Yang J-W, Ma W, Luo T, Wang D-Y, Lu J-J, Li X-T, et al. BDNF promotes human neural stem cell growth via GSK-3 β -mediated crosstalk with the wnt/ β -catenin signaling pathway. *Growth Factors*. (2016) 34:19–32. doi: 10.3109/08977194.2016.1157791
142. Yang J, Ru J, Ma W, Gao Y, Liang Z, Liu J, Guo J, et al. BDNF promotes the growth of human neurons through crosstalk with the Wnt/β-catenin signaling pathway via GSK-3β. *Neuropeptides*. (2015) 54:35–46. doi: 10.1016/j.npep.2015.08.005
143. Yue P, Gao L, Wang X, Ding X, Teng J. Intranasal administration of GDNF protects against neural apoptosis in a rat model of Parkinson's disease through PI3K/Akt/GSK3β pathway. *Neurochem Res*. (2017) 42:1366–74. doi: 10.1007/s11064-017-2184-1
144. Zhou Y, Zhu J, Lv Y, Song C, Ding J, Xiao M, et al. Kir6.2 deficiency promotes mesencephalic neural precursor cell differentiation via regulating miR-133b/GDNF in a Parkinson's disease mouse model. *Mol Neurobiol*. (2018) 55:8550–62. doi: 10.1007/s12035-018-1005-0
145. Chen X, Guo C, Kong J. Oxidative stress in neurodegenerative diseases. *Neural Regen Res*. (2012) 7:376–85. doi: 10.3969/j.issn.1673-5374.2012.05.009
146. Liu Z, Zhou T, Ziegler AC, Dimitrion P, Zuo L. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. *Oxid Med Cell Longev*. (2017) 2017:1–11. doi: 10.1155/2017/2525967
147. Thanan R, Oikawa S, Hiraku Y, Ohnishi S, Ma N, Pinlaor S, et al. Oxidative stress and its significant roles in neurodegenerative diseases and cancer. *Int J Mol Sci*. (2014) 16:193–217. doi: 10.3390/ijms16010193
148. Manoharan S, Guillemin GJ, Abiramasundari RS, Essa MM, Akbar M, Akbar MD. *Oxid Med Cell Longev*. (2016) 2016:8590578. doi: 10.1155/2016/8590578
149. Dalfó E, Portero-Otín M, Ayala V, Martínez A, Pamplona R, Ferrer I. Evidence of oxidative stress in the neocortex in incidental lewy body disease. *J Neuropathol Exp Neurol*. (2005) 64:816–30. doi: 10.1097/01.jnen.0000179050.54522.5a
150. Beal MF. Oxidatively modified proteins in aging and disease. *Free Radic Biol Med*. (2002) 32:797–803. doi: 10.1016/S0891-5849(02)00780-3
151. Brown GC, Borutaite V. Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. *Biochim Biophys Acta*. (2004) 1658:44–9. doi: 10.1016/j.bbabio.2004.03.016
152. Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. *J Parkinsons Dis*. (2013) 3:461–91. doi: 10.3233/JPD-130230
153. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol*. (2009) 7:65–74. doi: 10.2174/157015909787602823
154. Dringen R, Hirrlinger J. Glutathione pathways in the brain. *Biol Chem*. (2003) 384:505–16. doi: 10.1515/BC.2003.059
155. Galluzzi L, Blomgren K, Kroemer G. Mitochondrial membrane permeabilization in neuronal injury. *Nat Rev Neurosci*. (2009) 10:481–94. doi: 10.1038/nrn2665
156. Seet RCS, Lee C-YJ, Lim ECH, Tan JJH, Quek AML, Chong W-L, et al. Oxidative damage in Parkinson disease: measurement using accurate biomarkers. *Free Radic Biol Med*. (2010) 48:560–6. doi: 10.1016/j.freeradbiomed.2009.11.026
157. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet*. (2006) 38:515–7. doi: 10.1038/ng1769

158. Koziorowski D, Hoffman-Zacharska D, Sławek J, Jamrozik Z, Janik P, Potulska-Chromik A, et al. Incidence of mutations in the PARK2, PINK1, PARK7 genes in Polish early-onset Parkinson disease patients. *Neurol Neurochir Pol.* (2013) 47:319–24. doi: 10.5114/ninp.2013.36756
159. Friedman A, Arosio P, Finazzi D, Koziorowski D, Galazka-Friedman J. Ferritin as an important player in neurodegeneration. *Park Relat Disord*. (2011) 17:423–30. doi: 10.1016/j.parkreldis.2011.03.016
160. Schapira AH V. Mitochondrial dysfunction in neurodegenerative diseases. *Neurochem Res*. (2008) 33:2502–9. doi: 10.1007/s11064-008-9855-x
161. Gandhi S, Wood-Kaczmar A, Yao Z, Plun-Favreau H, Deas E, Klupsch K, et al. PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol Cell*. (2009) 33:627–38. doi: 10.1016/j.molcel.2009.02.013
162. Lotharius J, O'Malley KL. The Parkinsonism-inducing Drug 1-Methyl-4-phenylpyridinium triggers intracellular dopamine oxidation. *J Biol Chem*. (2000) 275:38581–8. doi: 10.1074/jbc.M005385200
163. González-Polo R, Soler G, Rodriguezmartin A, Moran J, Fuentes J. Protection against MPP+ neurotoxicity in cerebellar granule cells by antioxidants. *Cell Biol Int*. (2004) 28:373–80. doi: 10.1016/j.cellbi.2004.03.005
164. Wi S, Lee JW, Kim M, Park C-H, Cho S-R. An enriched environment ameliorates oxidative stress and olfactory dysfunction in Parkinson's disease with α-synucleinopathy. *Cell Transplant*. (2018) 27:831–9. doi: 10.1177/0963689717742662
165. Elokda A, DiFrancisco-Donoghue J, Lamberg EM, Werner WG. Effects of exercise induced oxidative stress on glutathione levels in Parkinson's disease on and off medication. *J Neurol*. (2010) 257:1648–53. doi: 10.1007/s00415-010-5584-6
166. Finaud J, Lac G, Filaire E. Oxidative Stress: relationship with exercise and training. *Sport Med*. (2006) 36:327–58. doi: 10.2165/00007256-200636040-00004
167. Chuang C-S, Chang J-C, Cheng F-C, Liu K-H, Su H-L, Liu C-S. Modulation of mitochondrial dynamics by treadmill training to improve gait and mitochondrial deficiency in a rat model of Parkinson's disease. *Life Sci*. (2017) 191:236–44. doi: 10.1016/j.lfs.2017.10.003
168. Costa RO da, Gadelha-Filho CVJ, Costa AEM da, Feitosa ML, Araújo DP de, Lucena JD de, et al. The treadmill exercise protects against dopaminergic neuron loss and brain oxidative stress in Parkinsonian rats. *Oxid Med Cell Longev*. (2017) 2017:1–10. doi: 10.1155/2017/2138169
169. Alkadhi KA. Exercise as a positive modulator of brain function. *Mol Neurobiol*. (2018) 55:3112–30. doi: 10.1007/s12035-017-0516-4
170. Archer T, Kostrzewa RM. Exercise and nutritional benefits in PD: rodent models and clinical settings. *Curr Top Behav Neurosci. (*2016) 29:333–51. doi: 10.1007/7854_2015_409
171. Monteiro-Junior RS, Cevada T, Oliveira BRR, Lattari E, Portugal EMM, Carvalho A, et al. We need to move more: neurobiological hypotheses of physical exercise as a treatment for Parkinson's disease. *Med Hypotheses*. (2015) 85:537–41. doi: 10.1016/j.mehy.2015.07.011
172. Tsou Y-H, Shih C-T, Ching C-H, Huang J-Y, Jen CJ, Yu L, et al. Treadmill exercise activates Nrf2 antioxidant system to protect the nigrostriatal dopaminergic neurons from MPP+ toxicity. *Exp Neurol*. (2015) 263:50–62. doi: 10.1016/j.expneurol.2014.09.021
173. Patki G, Lau YS. Impact of exercise on mitochondrial transcription factor expression and damage in the striatum of a chronic mouse model of Parkinson's disease. *Neurosci Lett*. (2011) 505:268–72. doi: 10.1016/j.neulet.2011.10.036
174. Koo J-H, Cho J-Y, Lee U-B. Treadmill exercise alleviates motor deficits and improves mitochondrial import machinery in an MPTP-induced mouse model of Parkinson's disease. *Exp Gerontol*. (2017) 89:20–9. doi: 10.1016/j.exger.2017.01.001
175. Al-Jarrah M, Obaidat H, Bataineh Z, Walton L, Al-Khateeb A. Endurance exercise training protects against the upregulation of nitric oxide in the striatum of MPTP/probenecid mouse model of Parkinson's disease. *NeuroRehabilitation*. (2013) 32:141–7. doi: 10.3233/NRE-130831
176. Aguiar AS, Duzzioni M, Remor AP, Tristão FSM, Matheus FC, Raisman-Vozari R, et al. Moderate-intensity physical exercise protects against experimental 6-hydroxydopamine-induced hemiparkinsonism through Nrf2-antioxidant response element pathway. *Neurochem Res*. (2016) 41:64–72. doi: 10.1007/s11064-015-1709-8

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REVIEW
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Diet in Parkinson's Disease: Critical Role for the Microbiome

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Background: Parkinson's disease (PD) is the most common movement disorder affecting up to 1% of the population above the age of 60 and 4–5% of those above the age of 85. Little progress has been made on efforts to prevent disease development or halt disease progression. Diet has emerged as a potential factor that may prevent the development or slow the progression of PD. In this review, we discuss evidence for a role for the intestinal microbiome in PD and how diet-associated changes in the microbiome may be a viable approach to prevent or modify disease progression.

Methods: We reviewed studies demonstrating that dietary components/foods were related to risk for PD. We reviewed evidence for the dysregulated intestinal microbiome in PD patients including abnormal shifts in the intestinal microbiota composition (i.e., dysbiosis) characterized by a loss of short chain fatty acid (SCFA) bacteria and increased lipopolysaccharide (LPS) bacteria. We also examined several candidate mechanisms by which the microbiota can influence PD including the NLRP3 inflammasome, insulin resistance, mitochondrial function, vagal nerve signaling.

Results: The PD-associated microbiome is associated with decreased production of SCFA and increased LPS and it is believed that these changes may contribute to the development or exacerbation of PD. Diet robustly impacts the intestinal microbiome and the Western diet is associated with increased risk for PD whereas the Mediterranean diet (including high intake of dietary fiber) decreases PD risk. Mechanistically this may be the consequence of changes in the relative abundance of SCFA-producing or LPS-containing bacteria in the intestinal microbiome with effects on intestinal barrier function, endotoxemia (i.e., systemic LPS), NLRP3 inflammasome activation, insulin resistance, and mitochondrial dysfunction, and the production of factors such as glucagon like peptide 1 (GLP-1) and brain derived neurotrophic factor (BDNF) as well as intestinal gluconeogenesis.

Conclusions: This review summarizes a model of microbiota-gut-brain-axis regulation of neuroinflammation in PD including several new mechanisms. We conclude with the need for clinical trials in PD patients to test this model for beneficial effects of Mediterranean based high fiber diets.

Keywords: Parkinson's disease, diet, microbiome, SCFA, LPS, intestinal hyperpermeability, vagus nerve, GLP-1

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INTRODUCTION

Parkinson's disease (PD) is recognized as the second most common neurodegenerative disease of aging after Alzheimer's disease (AD) and the most common movement disorder, affecting up to 1% of the population above the age of 60 and 4–5% of those above the age of 85 (1, 2). While there are treatments that minimize symptoms of PD, little progress has been made on efforts to halt disease progression (3). Less than 10% of PD is associated with specific genetic changes, which means that the search is on for environmental risk factors for PD (3, 4). Diet is one such environmental factor that has emerged as a potential factor that can promote the development or exacerbate the progression of PD (5–7). In this review, we will discuss evidence for the diet involvement in PD development, discuss the mechanisms by which the diet-mediated effects on the microbiome may influence PD, and also discuss how dietary interventions may be used to prevent or treat PD.

DIET IN PARKINSON'S DISEASE

There is a growing body of epidemiological evidence to support that diet impacts (positively or negatively) the development of neurodegenerative diseases such as PD. The Western diet is among the greatest risk factors for developing neurodegenerative diseases such as PD (8, 9). The Western diet is characterized by high caloric intake of energy dense foods, high in saturated and omega-6 (ω6) fatty acids, refined sugars, excessive salt intake, and low consumption of omega-3 (ω3) fatty acids and fiber (10–12). Studies of PD patients support total caloric intake of macronutrient and micronutrient correlate with symptom severity, with higher caloric intake associated with worse PD-related symptoms (13). Consumption of high quantities of animal saturated fat has been widely reported to be associated with increased risk of developing PD (14, 15). Foods associated with more rapid PD progression include canned fruits and vegetables, soda, fried foods, beef, ice cream, and cheese (all characteristic of the Western diet) (**Figure 1**) (9).

On the flip side, a "healthy" diet is associated with beneficial effects relative to PD (6). Adherence to the Mediterranean diet is associated with lower probability of developing PD (16). Specific components of the Mediterranean diet are particularly associated with these beneficial effects such as fresh vegetables, fresh fruit, nuts, seeds, non-fried fish, olive oil, wine, coconut oil, fresh herbs, and spices. Consumption of flavonoid-rich foods (tea, berry fruits, apples, red wine, and orange/orange juice) are also associated with a lower risk of developing PD (17). Polyunsaturated fatty acids (**PUFA**) are also inversely correlated with PD development (higher consumption of ω3 fatty acids is associated with reduced PD risk) demonstrating the influence of dietary fat intake on the brain (18, 19).

Diet can impact the body through multiple different mechanisms including direct effects of dietary components (e.g., vitamins, fats) on the body, but diet may modulate the development and/or progression of PD indirectly through effects on the intestinal microbiome (6, 20, 21). Indeed, diet is perhaps the single greatest factor determining the structure and metabolic function of the intestinal microbiota (22–25).

Coffee and caffeine in the diet have also been consistently associated with decreased risk of PD. Several key early studies showed a significant dose dependent decrease in risk for PD with increasing coffee consumption and for smoking as well (26–28). Recent studies have confirmed a decreased risk for PD in men and women with increasing caffeine consumption (29, 30). Both caffeine (coffee) and nicotine (smoking) have been shown to ameliorate disease in MPTP rodent models of PD (31, 32). In addition, coffee has recently been shown to contain chlorogenic acid that inhibits the NLRP3 inflammasome (33) and polyphenols that have been shown to be neuroprotective (34, 35) as well as promote healthy microbiome metabolism (36). Significantly, two recent reviews that discussed the beneficial effects of caffeine in reduced PD risk both propose a role for the microbiome (37, 38).

With regard to alcohol consumption and PD, there does not seem to be a clear conclusion. Two early large prospective studies showed no effect of moderate alcohol consumption and PD incidence (39, 40). However, another systematic review found a protective inverse relationship between alcohol use and PD (41). Another study found that heavy alcohol use was associated with decreased risk for PD (42). A recent review of all alcohol-PD studies concluded that prospective studies tended to find no association between alcohol use and PD with 2 studies finding an increased risk with moderate alcohol use and PD (43). However, the case-control studies were more likely to find a protective effect (43). Alcohol has also been shown to promote intestinal leakiness and microbiome effects (44–46). Thus, it appears there is no definitive view for the effects of alcohol consumption and risk for PD.

Consumption of dairy products is another area of diet that has evidence related to PD risk. Several studies have supported the view that high consumption of milk and possibly dairy products in general are associated with increased risk for PD (47–49). A diet study in Greece also found association of dairy and milk consumption with PD (50). Other more recent studies also supported association of dairy product consumption and increased PD risk (51, 52). A study in Hawaii found greater than two glasses of milk per day was associated with decreased neural density in the SN at autopsy (53). One proposed cause for these associations has been pesticides in the milk, but there is no data to support this. An intriguing recent study implicates microbiome bacteriophages, especially associated with *Lactococcus* bacteria in dairy products, as possible negative modulators of the bacterial gut microbiome in PD (54). However, a recent position paper on dairy products and PD risk concluded that overall the evidence did not warrant alarming the public to avoid dairy products (55).

There is considerable evidence that dietary or environmental exposure to neurotoxins such as rotenone and paraquat, maneb, and related neurotoxins such as MPTP can promote Parkinson's-like neurodegeneration (56, 57). All of these neurotoxins target the mitochondria and there is longstanding evidence that mitochondria dysfunction is critical in PD development (58, 59). Dysfunctional mitochondria activate the NLRP3 inflammasome (60). Both the herbicide paraquat and antifungal maneb have

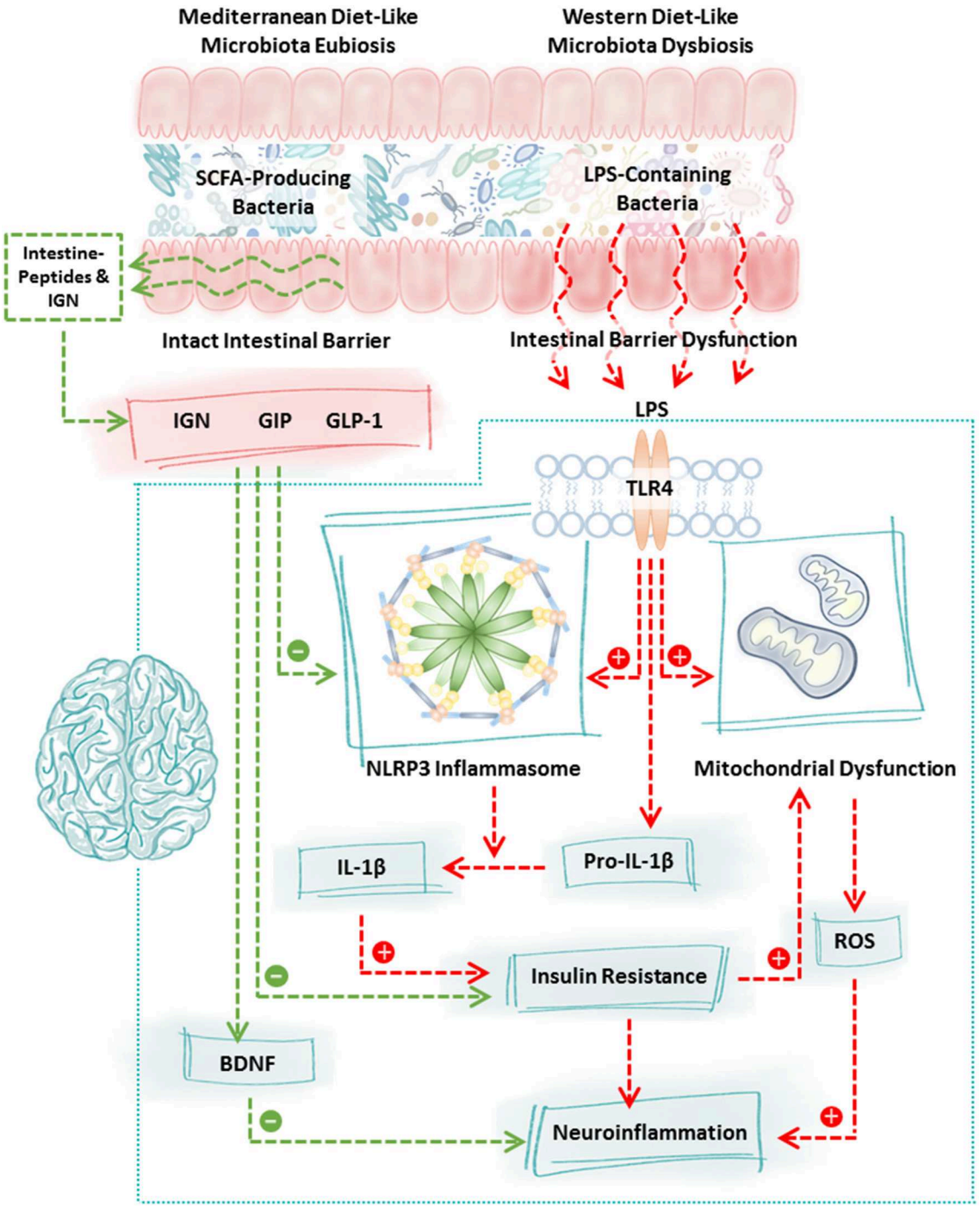

FIGURE 1 | Mechanisms of communication between the intestinal microbiota and the brain. Diet robustly impacts the intestinal microbiota. Consumption of a Western diet (or components of the Western diet) promotes the growth of LPS-containing bacteria and reduces the abundance of SCFA-producing bacteria whereas consumption of a Mediterranean diet (or components of the Mediterranean diet) promotes the growth of SCFA-producing bacteria and reduces LPS-containing bacteria. This shift is highly significant because LPS-containing bacteria are pro-inflammatory, they disrupt intestinal barrier integrity and LPS binding to TLR4 stimulates a cascade of events including NLRP3 inflammasome activation, mitochondrial dysfunction, and insulin resistance culminating in neuroinflammation and neurodegeneration. In contrast, increased production of SCFA due to consumption of the Mediterranean diet (or components of the Mediterranean diet) fortifies the intestinal barrier, stimulates the intestinal L-cell production of GLP-1 and GIP which inhibits NLRP3 inflammasome activation and normalizes insulin resistance. SCFA also stimulate intestinal epithelial cell IGN and together with GLP-1/GIP stimulate the vagus nerve and brain BDNF which has numerous beneficial effects on the brain and which improves neuron insulin resistance all of which function to promote neuronal health. Characteristic features of the PD microbiome are similar to those

(Continued)

FIGURE 1 | observed following consumption of the Western diet (low SCFA-producing bacteria, high LPS-containing bacteria); therefore, dietary interventions such as the Mediterranean diet (or components of the Mediterranean diet) may be a viable approach to blunt neuroinflammation and improve neuronal function in PD. BDNF, brain derived neurotrophic factor; GIP, gastrointestinal peptide; GLP-1, glucagon like peptide 1; IGN, intestinal gluconeogenesis; IL-1β, interleukin 1 beta; LPS, lipopolysaccharide; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3; ROS, reactive oxygen species; SCFA, short chain fatty acids; TLR4, toll-like receptor 4.

been linked to PD (56). Rotenone, a broad based pesticide, is currently used in animal models of PD (61, 62). MPTP, which also targets the mitochondria like the other neurotoxins listed, is also widely used as a model for PD (63, 64). There is a large body of epidemiological and experimental evidence for increased risk of PD due to environmental and dietary exposure to these neurotoxins (63–66). An early study found that exposure to pesticides resulted in a 70% increased risk for PD (67). These neurotoxins have been shown to cause Parkinsonian symptoms and SN neurodegeneration when injected systemically or directly into the striatum (62, 64). However, the effects of these environmental toxins on the microbiome has not been studied in depth. Significantly, in a PD mouse model of oral gavage administered rotenone, marked changes in the microbiome correlated with disease markers and TLR4 expression in the intestine and SN neuron loss (68, 69). Studies by this group also showed that a uridine and fish oil diet could ameliorate PD symptoms in these mice (61). In another rodent study using rotenone IP injection, changes were also found in the intestinal microbiome similar to those in PD patients (70). These studies support the model that both oral and systemic injection of these neurotoxins/pesticides can affect the microbiome. Another recent study showed that the pesticide diazinon could modulate the microbiome community in mice (71). Thus, the effects of these neurotoxins on the intestinal microbiome appears to be an important area for future study.

Recently the possibility of α-Syn in diet has become a focus of potential causes of PD (72). α-Syn is a 140 AA protein found in the brain as well as in lesser amounts in heart, muscle and other tissues and dairy products (72, 73). The function of α-Syn is unknown but hallmark inclusions known as Lewy pathology are found in neurons of the SN in PD and it is believed to play a role in PD (74). α-Syn aggregates can take many forms but it appears as though the fibrillar (PFF) form may be the most pathogenic in the brain because injection of this form can cause PD symptoms and pathology (75–77). Several studies support a possible spread of α-Syn with prion like properties and mutations in the α-Syn gene are associated with familial PD (78, 79). Recent experiments show injection of α-Syn PFF in the stomach or intestine traffic to the brain via the vagus nerve in rodents (80, 81). If α-synuclein spreads via a prion-like mechanism, then one question becomes, what are the origins of this prion-like species? One source could be meat products (72). First, it should be noted that no study has quantified the amounts of intact α-Syn in the stool. It may be that it is degraded by digestive functions and not available for uptake or absorption by the intestines. If it does remain intact, one possibility is α-Syn uptake by gut M cells. M cell depletion prevents oral prion infectivity (82). Also T cells in the gut and dendritic cells expressing LAG3 could bind α-Syn and promote its spread (83). Leaky gut could also be a mechanism for α-Syn translocation to the systemic circulation (84). Overall, research to date has yet to directly test the contribution of dietary α-synuclein to the mechanism of initiation and progression of PD (72). However, α-Syn is found in beef, pork, chicken, and fish and many people regularly consume these meat and dairy products, but only a small fraction of the general population will develop PD. Therefore, it is unlikely that eating meat products that contain α-Syn is an independent cause of PD (72). Nonetheless, future studies tracking α-Syn in the diet systemically as well as in the intestinal tract could provide new insights to a role for this key PD protein as a potential dietary risk factor.

DIET AND THE PD MICROBIOME

The human gastrointestinal tract (GIT) harbors trillions of microorganisms collectively referred to as the microbiome (24, 85, 86). We have a symbiotic relationship with the microbiota (the bacterial component of the microbiome). We provide them with an environment (the GIT) and food and they provide us with a myriad of benefits. The microbiota helps ward off harmful microorganisms (competitive exclusion), regulate immunity, and produce substances such as vitamins, secondary bile acids, and short chain fatty acids (SCFA) (24). For example, dietary fiber is used as a food source by the intestinal microbiota. Dietary fiber is a general term for consumed plant-based complex carbohydrates that are largely not digested by mammalian enzymes in the small intestine and consequently cannot be absorbed. However, they are available to be used as a food source by the intestinal (colonic) microbiota (87). Colonic bacterial fermentation of these dietary fibers generates metabolic byproducts and especially important are SCFA (10, 87–89). In contrast to these beneficial commensal bacteria, there are also pathogenic bacteria (pathobionts) that can cause GIT dysfunction (intestinal barrier dysfunction) and inflammation in the intestinal mucosa, systemic circulation, and even in the brain (10, 90). Thus, the balance of microbiota influences not only the GIT, but also organs throughout the body including the brain (91).

Although no two human microbiota communities are identical (influenced by lifestyle factors like diet, exercise, and genetics), recent studies in the last 10 years have shown people with certain diseases tend to share similar characteristic microbiota features (24, 92). An abnormal microbiome (so called "dysbiosis") is associated with many human diseases such as obesity/metabolic syndrome, inflammatory bowel disease (IBD) and other chronic inflammatory diseases as well as in PD (90, 93, 94). The intestinal microbiota has become a major focus of PD studies (95, 96). Initial studies by Scheperjans et al. (97) and Keshavarzian et al. (98) reported abnormal intestinal microbiota composition (dysbiosis) in PD patients. Subsequently, 15 additional studies from the USA, Europe and Asia have also

demonstrated dysbiosis in PD patients (**Supplementary Table 1**) (95, 96). As detailed in **Supplementary Table 1**, the PD patient's microbiota composition alterations are not identical in all of these studies. This is not surprising and should be expected because of the significant intra- and inter-individual variability discovered in the microbiota composition of healthy control subjects (99, 100) and other diseases, where intestinal dysbiosis has been reported (90, 92). Environmental factors, especially diet, can markedly affect microbiota community structure and composition, and thus it is expected that the intestinal microbiota in patients from the USA should be different from those living in Europe or Asia (21, 101). In fact, the intestinal microbiota was found to be significantly different in individuals living in different communities in the city of Chicago, Illinois, USA (102). The important key finding is that patients with PD have abnormal intestinal microbiota communities ("dysbiosis") regardless of where they live and also the PD microbiota community appears abnormal still after 2 years of follow up (103, 104). The majority of PD human studies employed bacterial 16S ribosomal RNA (rDNA amplicons) sequencing to different variable regions to identify bacteria in feces (majority of studies), colonic sigmoid mucosa (98), nasal wash (105) or nasal swab and oral (106, 107) samples. Three studies used targeted quantitative PCR, while one study utilized metagenomics shotgun sequencing. Regardless of sequence technique or bioinformatics methodologies, the overall common discovery indicated dysbiotic bacterial profiles, which suggested putative pro-inflammatory bacteria were more abundant and putative beneficial bacteria were less abundant in PD patients.

Parkinson's disease subjects demonstrated significantly altered intestinal microbial compositions in comparison to healthy controls with some overall trends worthy of comment. Briefly these include PD subjects to exhibit: increased relative abundance of genera *Akkermansia* (7 studies) (98, 105, 108–112), *Bifidobacterium* (5 studies) (103, 108, 110, 113, 114), and *Lactobacillus* (7 studies) (97, 103, 110, 111, 113, 115, 116); decreased abundance of genus *Prevotella* (7 studies) (97, 103, 104, 108, 109, 113, 117) and the family Lachnospiraceae (6 studies) (98, 103, 110, 111, 114, 117) along with its lower taxonomic hierarchal putative SCFA-producing genera *Faecalibacterium* (5 studies) (98, 108, 110, 113, 117), *Roseburia* (4 studies) (98, 103, 110, 111), *Blautia* (5 studies) (98, 103, 110, 113, 117), *Coprococcus* (2 studies) (98, 113), and *Dorea* (2 studies) (98, 113) (**Supplementary Table 1**).

Significantly, a few of the studies evaluated predicted functional gene content profiling (PICRUSt) (118) to infer changes in microbiota function. Keshavarzian et al. discovered PD subject's fecal samples had significantly higher abundant genes involved in lipopolysaccharide (LPS) biosynthesis, with a large number of genes involved in metabolism were significantly less abundant (98). Hill-Burns et al. indicated 17 upregulated pathways and 9 downregulated pathways, including xenobiotics degradation and metabolism of plant-derived compounds in PD subjects (110). Barichella et al. revealed 11 upregulated pathways and 15 downregulated pathways in *de novo* PD subjects, compared to healthy controls (111). Qian et al. predictive functional analysis indicated four metabolic pathways upregulated and 3 pathways downregulated (119). Finally, Bedarf et al. used the detailed metagenomics shotgun analysis to infer functional analyses of the metagenomes that showed differences in microbiota metabolism in PD subjects involving the β-glucuronate and tryptophan metabolism (109).

The intestinal microbiota does not appear to be the only microbiota that is disrupted in PD patients. To date, there are two studies that interrogated the nasal and oral microbiota community structure and composition in PD patients. Pereira et al. interrogated both nasal and oral microbiota profiles between PD patients and healthy controls (107). The oral microbiota composition was significantly altered in PD patients, compared to healthy controls, predominantly by higher relative abundance of opportunistic pathogens. The nasal microbiota lacked strong significant individual taxa differences, but trended toward an overall difference in the microbial composition between groups. In contrast, Mihaila et al. interrogated the oral microbiota using saliva samples through shotgun metatranscriptomic profiling and found significant changes in the microbiota community structure, composition and function in PD patients (106). The study found several similarities between dysbiotic oral microbiota and dysbiotic fecal microbiota in PD patients, when they compared their findings with previously published human PD studies. Dysbiotic oral microbiota once again was characterized by higher relative abundance of putative pro-inflammatory bacteria. This finding is potentially important in PD pathogenesis because one proposed site of initial injury in PD is the olfactory bulb, which is in close proximity to the oronasal space, as proposed by Braak et al. (120, 121).

However, the causal link between dysbiotic microbiota and the development of PD is yet to be established. The debate is whether these changes in microbiota community structure and composition in PD starts the trigger for PD, or are a consequence of PD. Indeed, several studies have shown a correlation between changes in microbiota and duration of the disease and dysbiosis is more pronounced in those with longer duration of PD (98, 104, 115, 117). This is not surprising because PD patients commonly change their life habit to better cope with their symptoms and this life style change can impact microbiota composition. For example, GI symptoms are common in PD patients (122, 123) and thus they typically change diet that could affect their microbiota. Although several studies did not find a correlation between diet and dysbiosis in PD patients numerous studies support a role for Western diet and possibly dairy products in PD risk (47, 51, 61, 124, 125). Constipation is very common in PD patients and typically occurs years before onset of CNS symptoms (123, 126, 127) and constipation can impact the microbiota community (128). However, dysbiosis was also found to occur in those PD patients who did not suffer from constipation (98). Patients with PD have poor sleep and reversal of sleep/wake cycles that can cause disruption of circadian rhythms (129, 130) and both disrupted sleep and circadian disruption can cause dysbiotic microbiota in both humans and rodents (131, 132). Additionally, PD medication correlates with dysbiosis (105, 110, 119). However, dysbiosis was still present in early onset and naïve PD patients on no PD medication (98). More importantly, dysbiosis has been reported

in patients with idiopathic rapid eye movement sleep disorder (iRBD) (prodromal PD) (105). Thus, even though life style changes from PD symptoms and PD medication may contribute to changes in microbiota composition, it does not appear to explain the observed dysbiosis in PD patients. Taken together, these findings support the hypothesis that abnormal microbiota composition plays a critical role in the pathogenesis of PD and is a major contributor of symptomatic PD development.

One key question is how does the intestinal microbiota dysbiosis observed in multiple PD studies arise? The current model for a role for the microbiome in PD is that dysbiosis may be driving PD progression either via systemic inflammatory factors and/or increased α-Syn misfolding in the gut that results in aggregates of α-Syn being transported to the brain via the vagus nerve as hypothesized by Braak (95, 120). However, there is no established mechanism to explain the intestinal microbiome dysbiosis or even to what extent it is a consequence or cause of PD (95, 96, 133). Studies in which PD patient fecal transplant into genetic PD mice worsened the PD phenotype support a role for microbiome dysbiosis directly promoting PD progression (134). One possibility is a genetic contribution considering that LRRK2 polymorphisms are associated with PD risk and IBD risk and LRRK2 mediates microbial immune signaling. But the majority of sporadic PD appears to be associated with environmental risk factors that also affect the microbiome such as stress, diet, lack of exercise, and disruption of circadian rhythms seen in REM sleep behavior disorder (RBD) (4, 105, 135–137). Change in life style with PD that helps patients to cope with symptoms can affect microbiota-like lack of exercise and change in diet- these changes can explain worsening of dysbiosis in those with a long duration of PD but also in early onset PD (138). Gut dysfunction also can affect microbiota and constipation (128) could be a contributing factor and several studies link prodromal constipation with PD (123, 126). However, constipation cannot explain dysbiosis completely because PD patients without constipation still had dysbiosis and leaky gut (84, 98). Also, PD patients with RBD who had no constipation still exhibited dysbiosis (105). Thus, while the search goes on for mechanisms for PD dysbiosis, the most likely cause is Western lifestyle factors known to affect the microbiome including stress, Western diet, lack of exercise, and circadian disruption (4, 136).

MICROBIOTA-GUT-BRAIN AXIS IN PD

Recent models for PD pathogenesis have focused on the important role of the microbiota-gut-brain axis (MGBA). One school of thought, originally proposed by Braak et al. (120) actually proposes that PD originates in the GIT or possibly the nasal mucosa (121) and spreads to the brain (139, 140). In support of this model several studies have shown α-Syn protein exhibits prion-like properties and cell to cell transmission (141). Key papers showed inter-neuronal trans-synaptic transport of α-Syn in pathological studies in PD patients that had received striatal transplants supporting the spread of misfolded α-Syn to normal adjacent cells (142–144). Recent studies have now shown EE cells of the gut can produce misfolded α-Syn and synapse with enteric nerves to transmit α-Syn (14, 145). However, the true role of α-Syn in PD is still debated (146). The role of α-Syn in the intestine is discussed further below. The MGBA is the two-way communication between the GIT and the CNS/brain and consists of many mechanisms (6, 147, 148). The mechanisms of communication used by the MGBA include responses to bacterial components and bacterial metabolites (including pro-inflammatory products like LPS that could activate microglia and trigger neuro-inflammation) (149–151) and anti-inflammatory products like SCFA, especially butyrate (152), peptides [including neurotransmitters and neuromodulators such as g-aminobutyric acid (GABA)], serotonin, dopamine (151, 153) and hormones produced by cells of the GIT (154, 155). This interaction includes bidirectional microbiota–immune interaction and microbiota-nervous system interaction. In fact, a growing number of studies support two-way interaction of the microbiota with virtually every organ system (24). This bidirectional communication is increasingly acknowledged as playing an important role in brain function including in neurodegenerative diseases (147, 151).

Evidence supports that virtually every part of the GIT is affected in PD (122, 123). A pathologic hallmark of PD are so called Lewy bodies in the brain substantia nigra (SN) neurons that are found post-mortem. Lewy bodies are largely composed of the neuronal protein alpha synuclein (α-Syn). A key feature in PD is that aggregated and phosphorylated forms of α-Syn protein have also been observed in every major part of the GIT and enteric nervous system in patients with PD (84, 123, 156–158). For example, Lewy bodies/Lewy neurites are present in 72–100% of intestinal samples from PD subjects and 62% have phosphorylated α-Syn which is markedly greater than that observed in the healthy population (0–33% have α-Syn). These data suggest that intestinal synucleinopathy may be a relatively sensitive and reliable indicator of PD (123, 159). Importantly, increased phosphorylated α-Syn is also found in GIT tissues from prodromal PD patients suggesting that GIT involvement occurs early in disease pathogenesis (159, 160). This is supported by a recent study which reported that distinctive α-Syn immunoreactivity observed in intestinal biopsies collected from healthy individuals who would later go on to develop PD (156, 157). Taken together, these data support the idea that abnormal enteric α-Syn appears before neurodegeneration in CNS advances to a point that is sufficient for motor symptoms to emerge. Such data also support an intestinal origin for PD.

Motor impairments in PD are generally preceded by non-motor symptoms such as depression, olfactory deficits, sleep behavior disorder, and a number of GIT symptoms. The GIT symptoms can precede motor symptoms by more than 10 years and include GIT motility problems, colonic inflammation, and constipation (50–80%) (123, 127, 161). In fact, constipation is associated with a 2.7- to 4.5-fold increase in the risk of developing PD (123).

In 2003, Braak et al. postulated that an unknown pathogen (virus, bacterium) or toxin originating in the GIT or nasal passage/olfactory nerve (two hit hypothesis) could be responsible for the initiation of sporadic PD (120, 121). In this model of disease progression, the pathology initiates in the GIT (or nasal/olfactory) and propagates to the brain via the Vagus nerve

or olfactory nerve (120, 162). Researchers have demonstrated that α-Syn fibrils, injected into the GIT mucosa of rodents, can propagate through the Vagus nerve and can be found in the brain (81, 163). Another recent study injected pre-formed α-Syn fibrils into the mouse stomach mucosa and found progressive PD pathology including α-Syn misfolding in the Vagus nerve and SN, an effect that was absent in vagotomized mice (80). With regard to vagotomy and risk of PD, a study by Svensson et al. found that full truncal vagotomy is associated with a decreased risk for subsequent PD, supporting that the vagal nerve may be critically involved in the pathogenesis of PD as proposed by Braak et al. (120, 164). However, two subsequent studies have disputed these findings. Tysnes et al. reanalyzed these data and found no significant risk reduction for PD with vagotomy (165). In addition, a second independent human study in Sweden found no decreased risk for PD after vagotomy (166). Thus, vagal involvement in PD disease development is still disputed (133).

However, even if the Vagus nerve isn't critical in initiating or promoting α-Syn PD pathology there are many other mechanisms by which the GIT can impact the brain via the vagus as we discuss below. The changes observed in the GIT in humans and animal models of PD are intriguing and begs further investigation into what is causing the GIT dysfunction and α-Syn aggregation to occur (140). One possible factor is the intestinal microbiota (95, 96).

A growing body of evidence now supports that the intestinal microbiota modulates behavior and contributes to neurological disorders and neurodegenerative diseases (151, 167–169). In fact, data show that the intestinal microbiota is necessary for the development of PD-like behavior and pathology in rodent models. Specifically, germ-free mice and antibiotic-treated mice have ameliorated PD-like behavior and pathology compared to their specific pathogen free counterparts (134). These data suggest that signaling between the microbiota and the brain is critical for PD-like outcomes in rodent models. It also appears that there is something remarkable about the PD microbiome that triggers events leading to neuroinflammation and neurodegeneration. Transfer of a microbiome from an MPTP-treated mouse into a control (non-MPTP) mouse is sufficient to induce motor impairment and activation of microglia and astrocytes in the SN (170). In addition, colonization of α-Syn-overexpressing (ASO) mice with microbiota from human PD patients enhances motor impairments compared to mice that received microbiota transplants from healthy human donors (134). These findings support that intestinal microbiome can regulate the development of PD-like pathology and behavior in mice and therefore may also be important in contributing to disease development in humans (95, 96). Perhaps PD should no longer be viewed solely as a complex disorder of motor functions, but rather as a progressive condition involving the GIT (6, 148, 171).

GIT-derived bacteria, bacterial components, and bacterial metabolites can trigger neurodegeneration through multiple pathways which are affected by diet and discussed below. First, is the intestinal barrier mechanism. In this mechanism, bacterial components (e.g., LPS) and bacterial metabolites (e.g., SCFA) produced by the microbiota influence intestinal barrier integrity which directly contributes to inflammation in the systemic circulation and in the brain (91, 137, 172). Second, is the NLRP3 inflammasome activation mechanism. Endotoxemia (i.e., LPS in the blood) resulting from barrier dysfunction activates the NLRP3 inflammasome and results in mitochondrial dysfunction and IL-1b production and insulin resistance with important consequences for neuronal function (77). Finally, are the intestinal peptide and intestinal gluconeogenesis mechanisms (173, 174). Bacterial metabolites influence the production of the GIT peptide production, insulin resistance, mitochondrial function, and vagal stimulation of brain derived neurotrophic factor (BDNF) production in the brain. This list of potential mechanisms is by no way means exhaustive but reflects key topics that are rapidly emerging as factors contributing to diet-microbiome regulation of gut-derived inflammation in neurodegeneration and PD.

INTESTINAL BARRIER MECHANISM

The intestinal epithelial barrier separates the pro-inflammatory luminal contents (e.g., LPS) from reaching the intestinal and systemic circulation, and the intestinal microbiota is a critical regulator of intestinal barrier integrity (91, 175). Intestinal barrier dysfunction (i.e., intestinal leakiness) has been observed in newly diagnosed, untreated PD patients which is also associated with increased LPS staining and α-Syn aggregates in the colonic mucosa (84). GIT dysfunction has also been described in animal models of PD including in both genetic and toxin-induced models (122, 123) which occurs concurrently with α-Syn aggregations in the GIT (123). These observations further support the hypothesis that PD may originate in the GIT (139).

Indeed, intestinal microbiota dysbiosis (especially when characterized by a reduction in SCFA-producing bacteria that has been reported in PD patients) is associated with intestinal barrier dysfunction and endotoxemia (i.e., LPS in the blood) (91, 95). Specifically, bacterial production of SCFA appear to be critically important in regulating the barrier (87). The three principal colonic SCFA include acetate (2-carbon), propionate (3-carbon), and butyrate (4-carbon). These typically exist in the colon in a millimolar ratio of 60:20:20 (acetate:propionate:butyrate) (176). Two other important SCFA receiving are lactate and succinate. SCFA exert beneficial effects through multiple mechanisms (87). Previous reviews of SCFA mechanisms have focused on SCFA specific GPCR signaling via specific receptors: GPR41 (propionate/butyrate), GPR43 (acetate/propionate), and GPR109a (butyrate) for acetate, propionate, and butyrate (87, 177). Also GPR81 (lactate) and GPR91 (succinate) have received recent attention (87). These GPCR for SCFA are reviewed in detail elsewhere (87, 177). Broadly speaking, SCFA GPCR positively modulate immunity and anti-inflammatory signaling in immune and other cells as well as mitochondrial cellular metabolism (178, 179). Butyrate (and to a lesser extent propionate and acetate) also has histone deacetylase inhibitor (HDACi) activity that can have epigenetic effects on gene expression, and butyrate is used by colonocytes as an energy source (10, 177). It is through

these mechanisms that SCFA (especially butyrate) influences intestinal barrier integrity. Indeed, a reduction in putative SCFA-producing bacteria or a reduction in luminal SCFA (due to intestinal microbiota dysbiosis) is associated with intestinal barrier dysfunction (10, 87, 180).

Diet-induced dysbiosis or even age-associated dysbiosis (a normal feature associated with aging) (91, 175), are characterized by a loss of SCFA-producing bacteria and SCFA, these may be able to trigger intestinal barrier dysfunction and subsequent inflammatory events leading to systemic inflammation as well as neuroinflammation and neurodegeneration (101, 175). Newly diagnosed, treatment naive PD subjects have evidence of intestinal barrier dysfunction compared to age matched controls (84, 181). Specifically, PD subjects have elevated levels of serum LPS binding protein (LBP, binds to LPS to elicit an immune response), abnormal intestinal tight junction proteins, fecal markers of leaky gut, serum zonulin, as well as *E. coli* in the intestinal mucosa compared to age matched controls (84, 181, 182). In support of intestinal barrier dysfunction being a critical mechanism, diseases characterized by intestinal microbiota dysbiosis and barrier dysfunction are a risk factor for developing PD. Specifically, Four studies in patients with inflammatory bowel disease (IBD), which is also characterized by intestinal microbiota dysbiosis and barrier dysfunction, support a significantly increased risk for developing PD compared to people without IBD (183–186). Also, a recent systematic review and meta-analysis of these four IBD-PD studies above concluded that the overall risk of PD in IBD was significantly higher than controls. Crohn's disease had a 28% increased risk of PD and ulcerative colitis had a 30% increased risk of PD compared to controls (187). In support of these data two studies using the DSS rodent model of ulcerative colitis concluded that DSS in drinking water and the resulting intestinal inflammation exacerbated symptoms of PD in both the LPS-striatum injection PD model (188) and an α-Syn overexpressing genetic PD model (189). However, in one recent US study using a large Medicare database analysis and newly diagnosed PD patients, IBD was associated with lower risk of PD as were Crohn's disease and Ulcerative colitis individually (190). The reasons for these differences are not clear and the role of IBD in PD risk remains to be defined. Studies have also demonstrated that a genetic variant that is a risk factor for IBD (leucine rich repeat kinase 2, LRRK2, important in the response to microbial ligands), is also a risk factor for PD (191). Furthermore, restraint stress (which caused intestinal barrier leak) exacerbated PD-like symptoms and loss of dopaminergic neurons in the striatum in the rotenone rodent model of PD (137).

Endotoxin in the blood (as a consequence of intestinal barrier dysfunction) can affect the brain directly (101, 149, 175). Like PD, Alzheimer's disease (AD) is a neurodegenerative disease that is also characterized by intestinal microbiota dysbiosis and barrier dysfunction (192, 193). Recent post mortem analysis of AD patient brains reveals LPS staining in the hippocampus and cortex of AD patients is 21-fold greater than that observed in control brain tissue (150, 194). Like AD, PD is also characterized by intestinal barrier dysfunction and endotoxemia (84), therefore it is possible that intestinal barrier dysfunction may play a key role in PD development and/or progression (95).

Mechanistically, Western diet dysbiosis, intestinal barrier dysfunction and endotoxemia can lead to immune activation and neuroinflammation (91, 101, 195–197). Toll like receptors (TLRs) recognize pathogen associated molecular patterns (PAMPs) located on the surface of bacteria (198). Among the most widely studied is the interaction between TLR4 and LPS (199). TLRs are located on a wide variety of cell types and are critical to mount an appropriate immune response to bacteria. In fact, administration of systemic LPS has been used as a model for PD for many years (149, 197, 200). Mechanistically, this appears to be the consequence of LPS-driven activation of TLR4, especially on brain microglia (201). Specifically, TLR4 knock out mice are protected from the effects of oral low dose rotenone as well as MPTP including less neuroinflammation and neurodegeneration, compared to rotenone-treated, wild-type mice (68, 202). These data support that TLR4 receptors are important in the development of PD-like pathology.

Taken together, it appears that barrier dysfunction, leading to endotoxemia, and TLR4 receptor activation may result in a series of events culminating in systemic inflammation and neuroinflammation and neurodegeneration (91, 101, 203–205). Even if intestinal barrier dysfunction is a consequence of PD (and not an initiating trigger/cause), intestinal barrier dysfunction and the resulting endotoxemia may still produce sustained neuroinflammation that promotes PD disease progression (101, 203).

NLRP3 INFLAMMASOME ACTIVATION MECHANISM

One of the consequences of TLR activation is microglial NLRP3 inflammasome activation (77, 206). In response to activation of TLRs, the NLRP3 inflammasome assembles and produces inflammatory cytokines (207, 208). Among the most widely studied inflammasomes is the NLRP3 inflammasome which produces pro-inflammatory cytokines especially IL-β as well as IL-1α, IL-18, and IL-33 (209). Inflammasomes are present in peripheral immune cells such as macrophages, as well as in the brain and especially in microglia (206, 210, 211). A role for microglial NLRP3 inflammasome in PD has recently been proposed (77). The NLRP3 inflammasome has also emerged as a potential driver of α-Syn neuroinflammation in PD (212). The current model of NLRP3 activation proposes a "two signal" model (213). In this model, TLR signaling is the first signal which induces NF-kB-mediated expression of pro-IL-1β and pro-IL-18. The second signal can be ATP, calcium or potassium flux or mitochondrial reactive oxygen species (**ROS**) which can occur as a consequence of a number of factors such as intestinal microbiota dysbiosis, endotoxemia (11, 213–217) or other factors that induce mitochondrial dysfunction such as aging (60, 218). Another possible second signal is misfolded α-Syn (aggregated α-Syn) that was induced by TLR/NF-kB mediated inflammation (77, 212). The second signal induces NLRP3 assembly and subsequent caspase-1 activation. The combination of the first

and the second signals results in cleavage of pro-IL-1β to its active form IL-1β (and other cytokines like IL-18) (213) which has a wide range of biological consequences including creation of sustained pro-inflammatory/oxidative stress in the brain that would lead to more α-Syn aggregation, more neuroinflammation enough to cause DA loss and neurodegeneration and symptoms of PD (77).

There is a substantial amount of data demonstrating the importance of the NLRP3 inflammasome in PD. Recent post mortem studies in PD patients show that the NLRP3 inflammasome is significantly upregulated in the SN of PD patients (almost entirely localized to microglia) (77). This upregulation in NLRP3 was also observed in mouse models of PD and AD (77, 219) and it appears to be important in disease pathogenesis. Specifically, inhibition of NLRP3 protects against neurodegeneration in all rodent models of PD tested including injection of pre-formed α-Syn fibrils (PFF), rotenone, and MPTP models (77, 215, 220). Similarly, knocking out NLRP3 in an AD animal model (another neurodegenerative disease) protects mice from developing AD-like behavior and brain pathology (219). Thus, activated NLRP3 inflammasome appears to be a key driver of neuroinflammation in PD (77, 220). In addition, NLRP3 levels also appear to increase with other factors such as age and consumption of a Western diet, it could be that the increase in NLRP3/IL-1b reduces the resiliency of the brain to respond to a secondary insult such as gut-derived endotoxemia from microbiota dysbiosis and/or intestinal barrier dysfunction (11, 221, 222).

In addition to LPS activation of TLR4, the microbiota can also influence the NLRP3 inflammasome by producing secondary bile acids. Primary bile acids are produced in the liver and are subsequently released into the GIT to aid in the digestion and absorption of lipids. Most primary bile acids are absorbed in the small intestine but those that reach the colon are metabolized by the intestinal microbiota to form secondary bile acids. Importantly, secondary bile acids can inhibit the NLRP3 inflammasome via the TGR5 receptor and are dysregulated in Western diet induced dysbiosis (223, 224).

As already mentioned, NLRP3 activation results in production of several cytokines but perhaps the one that may be most relevant for PD is IL-1β. IL-1β is not only a potent pro-inflammatory cytokine and thus a major player in neuroinflammation in PD, but also has many other biological effects. Among the many consequences of IL-1β production is the development of insulin resistance (218). Specifically, IL-1β blocks signaling associated with insulin receptors. Activation of NLRP3 and subsequent IL-1β production are the single greatest factors that drive insulin resistance, and NLRP3 KO mice are protected from developing insulin resistance (225, 226). Specifically, cytokines, especially IL-1β, block insulin signaling which has important detrimental consequences on neuronal mitochondrial function and cellular health. In fact, insulin resistance is characteristic of both the PD and AD brain (227–229) and diabetes is a risk factor for development of PD (228).

Insulin resistance and type 2 diabetes mellitus (T2DM, characterized by insulin resistance) may cause neuroinflammation by driving mitochondrial dysfunction, leading to excessive production of ROS, cellular stress, NLRP3 activation and neuroinflammation (especially via microglia), ultimately culminating in neuronal dysfunction and death (228, 229). Insulin resistance is commonly observed during aging, but it may also be important in the pathogenesis of PD (229). The incidence of both T2DM and PD are both increasing in Western societies suggesting that these two diseases may be related (230). In fact, as noted, T2DM is a risk factor for PD and is characterized by intestinal microbiota dysbiosis similar to that observed in PD (loss of SCFA-producing bacteria, increase in LPS-containing bacteria) (231–236). Premature cognitive decline is also a feature commonly observed in patients with T2DM (231). Inhibition of NLRP3 (via glyburide or pioglitazone, the SCFA butyrate, or MCC950) prevents the development of insulin resistance and T2DM as well as PD (77, 211, 237–240). Taken together, these data support a model for a cascade of events culminating in intestine-derived neuroinflammation and neurodegeneration. Specifically, LPS-TLR activation of the NLRP3 inflammasome induces production of IL-1β resulting in insulin resistance, mitochondrial dysfunction, and ROS production, further NLRP3 activation and neuroinflammation and neurodegeneration.

INTESTINAL PEPTIDE AND INTESTINAL GLUCONEOGENESIS MECHANISMS

Influence of diet and the intestine on brain function (gut-brain axis) is not necessarily limited through intestinal microbiota. The intestine produces a number of substances that directly or indirectly influence the brain. These substances are produced in response to dietary components (e.g., fats) but also are produced in response to bacterial metabolites. Bacterial products, SCFA and secondary bile acids, can both promote the production of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) by L-cells of the GIT (87, 241–243). GLP-1 and GIP impact a number of cell types that can directly or indirectly affect neuroinflammation and neurodegeneration in PD.

GLP-1 has multiple mechanisms of action. One important consequence of GLP-1 production is reduced inflammation. For example, stimulation of the GLP-1 receptor (via GLP-1 or agonists) inhibits the NLRP3 inflammasome (244–246). In so doing, GLP-1 prevents the cascade of events including IL-1β production culminating in insulin resistance, mitochondrial dysfunction and cellular stress. GLP-1 also corrects insulin resistance by stimulating pancreatic cells to produce insulin and normalizing insulin signaling and mitochondrial function in brain neurons (247). Normalizing insulin resistance improves mitochondrial function and reduces ROS production, which has the net effect of blocking neuroinflammation and improving neuronal health. GLP-1 can have effects within the brain itself because it can cross the blood brain barrier and receptors for GLP-1 are located on neurons, astrocytes, and microglia (247–249). GLP-1R-deficient mice show impaired performance in memory-related behavioral tasks (248). In addition, GLP-1 is protective against neuronal apoptosis in the Alzheimer's

disease model (247). Finally, stimulation of GLP-1 receptors induce production of BDNF in the brain and also stimulate vagal signaling from the gut to further promote brain BDNF (247, 248). BDNF is a critical factor for survival and health of dopaminergic neurons in the SN (250). Indeed BDNF is dramatically decreased in PD brain tissue, thus, the ability to increase BDNF is an important consequence of GLP-1 production (250, 251).

Alterations in GLP-1 signaling are associated with many features associated with PD or risk factors for developing PD. For example, intestinal microbiota dysbiosis disrupts normal GLP-1 signaling (252), reduced GLP-1 production is associated with metabolic syndrome (insulin resistance) (253), and reduced GLP-1 is associated with reduced BDNF in the brain (254). On the flip side, GLP-1 agonists are protective in several rodent models of PD (174, 247). Agonists of GLP-1 and dual treatment of GLP-1/GIP demonstrate neuroprotection in MPTP models of PD (255, 256). It is possible that these effects are mediated through a mechanism involving both inhibition of NLRP3 and an increase in the production of glial derived neurotrophic factor (GDNF) and BDNF and may involve GLP-1 induced improvement in insulin sensitivity as well as GLP-1 vagal stimulation (174). Importantly, recent clinical trials show that GLP-1 agonists elicit significant improvements in PD patient disease scores compared to placebo (248, 257, 258).

Intestinal gluconeogenesis (IGN) is also a mechanism by which the diet and microbiota can influence neuroinflammation and neurodegeneration. Recent studies have shown that the SCFA (butyrate, propionate) can regulate host metabolism by stimulating IGN in intestinal epithelial cells that in turn promotes vagal signaling (173). It should not be surprising then that a healthy high fiber diet and increased gut SCFA can correct insulin resistance via both IGN-vagal-BDNF signaling and by GLP-1/GIP stimulation and preventing intestinal leakiness and NLRP3 activation (10, 87, 259). IGN vagal BDNF stimulation is a key mechanism by which IGN may promote normal brain glucose metabolism which is dysregulated in PD (173). Thus, IGN from gut SCFA can also influence BDNF production in the brain via the vagus (260). BDNF promotes neuronal cell health and normal insulin signaling in the brain (261). It makes sense then that impaired insulin sensitivity in the PD brain is associated with low BDNF levels (250, 262–264).

There are multiple mechanisms by which GLP-1, GIP, and IGN can influence the brain but it is interesting that they all share the feature of being able to upregulate production of BDNF (262). BDNF is also a key neurotrophic factor in CNS degeneration and regeneration (262). Reduced levels of serum BDNF are observed in PD patients compared to healthy controls, including in the serum and in the brain (SN, caudate-putamen) (251, 265, 266). It is intriguing to think that Western diet intestinal microbiota dysbiosis leading to low SCFA production might blunt the expression of BDNF through a mechanism involving gut leakiness and loss of GLP-1, GIP, and/or IGN. Western diet dysbiosis also results in loss of (fewer) gut vagal afferents in rats (267). Finally, it is noteworthy that GLP-1, GIP, and IGN and other intestinal hormones are largely influenced by diet and dietary intervention such as switching from primarily animal-based Western diet to primarily plant-based diet can promote normal homeostasis of these hormones. These data are yet another scientific rationale for considering dietary intervention to prevent/treat or at least modify disease course in PD.

DIET AS A PREVENTION OR TREATMENT FOR PD

Based on these data it is clear that there are several mechanisms by which intestinal bacteria, bacterial products, or bacterial metabolites and intestinal hormones can influence neuroinflammation and neurodegenerative processes. Therefore, it seems logical that dietary interventions targeted at modifying the intestinal microbiota structure and/or function and intestinal peptides may modify PD disease pathogenesis. Indeed, Hippocrates' said: "Let food be thy medicine and medicine be thy food" (10). Diet has recently gained importance as a risk factor for developing PD and also as a potential therapeutic approach to treat PD (6, 7, 268). Below is a summary of dietary interventions that may be useful in the prevention and/or treatment of PD as well as the mechanisms by which this benefit may be conferred on the brain.

MEDITERRANEAN DIET AS A TREATMENT

The main components of the Mediterranean diet (MedD) include: daily consumption of vegetables, fruits, nuts, whole grains, and healthy fats; weekly consumption of fish, poultry, beans, and eggs; moderate consumption of dairy products; and limited intake of red meat (10, 124). Adherence to the MedD is associated with decreased risk of PD (9, 269, 270). One of the most dramatic differences between the traditional Western diet and the MedD is dietary fiber intake. Consumption of dietary fiber is typically very low (<10–15 g/day) in Western societies, but high (>25–30 g/day) in those who consume a Mediterranean diet (10, 87–89). It makes sense then that the Mediterranean diet-associated microbiome is characterized by a high relative abundance of bacteria that can utilize fiber as an energy source such as SCFA-producing bacteria (10, 89). Indeed, microbiota communities from subjects consuming a Mediterranean diet are enriched in SCFA-producing bacteria (10, 87, 89, 271). Fiber can also be administered experimentally to alter the microbiota structure and function including an increase in the relative abundance of fiber-fermenting ("good") bacteria as well as increased production of SCFA (10, 87).

These microbiome changes can elicit a myriad of effects that are beneficial in blunting neuroinflammation and PD pathogenesis. For example, consumption of a high fiber diet improves intestinal barrier function and insulin resistance, improves insulin sensitivity, increases GLP-1/GIP production, stimulates IGN, and increases brain BDNF production (173, 259, 272, 273). Conversely, when fiber consumption is low, the microbiota instead use protein as an energy source which favors the growth of gram negative (LPS-producing, dysbiosis) bacteria and the production of metabolites such as branched chain fatty acids including isovalerate and 2-methyl butyrate that have been associated with insulin resistance (a feature of PD) (274). Fiber

consumption (and the consequent production of SCFA) is one mechanism by which the Mediterranean diet may beneficially impact PD development and progression.

In addition to fiber, the Mediterranean diet is also rich in foods that contain anti-oxidant bioflavonoids and polyphenols, which are associated with decreased risk of PD (9, 35, 270). Flavonoids are typically found in fruits, vegetables, grains, and tea. There are not a lot of data available, but it appears that flavonoid consumption also may trigger an increase in SCFA production (36) and several polyphenol bioflavonoids (including in coffee) and fish oil are associated with inhibition of the NLRP3 inflammasome (33, 275). Also, nuts and olive oil stimulate GLP-1 secretion and the MedD after 28 days has been shown to increase GLP-1 production (241).

Taken together there are multiple mechanisms by which the Mediterranean diet can beneficially impact the brain. There is a common theme that components of the Mediterranean diet are especially able to alter the microbiota in a way that promotes SCFA production. SCFA can influence so many PD relevant mechanisms such as barrier function, mitochondrial function, NLRP3, and intestinal peptide production (259, 272, 273) and vagal stimulation of BDNF and thus might be beneficial in PD. However, to date there is no high-quality clinical trial to test the potential benefit of a high fiber Mediterranean diet in PD. These data above provide a strong scientific rationale for conducting randomized controlled dietary trials in PD to determine whether Mediterranean diet can impact neuroinflammation and disease course of PD patients.

KETOGENIC DIET AND FASTING AS A TREATMENT

It is well-established that caloric restriction and/or intermittent fasting are anti-inflammatory processes and can ameliorate disease in a variety of experimental models, including PD (276, 277). Intermittent fasting is a feeding regimen that cycles between periods of fasting (with either no food or significant caloric restriction), and periods of unrestricted eating. Caloric restriction can improve health, increase lifespan, and improve tolerance to metabolic stresses (278, 279). Indeed, rodents on an intermittent fasting diet exhibit less neuronal dysfunction/degeneration, and fewer PD-like symptoms in models of PD compared to *ad libitum*-fed controls (280). Similarly, caloric restriction increases levels of neurotrophic factors such as BDNF and attenuates PD-like pathology (including dopaminergic neuron loss) and behavior in rodent and primate models of PD (281, 282) lifestyle interventions such as caloric restriction/fasting and ketogenic diets are currently used to treat epilepsy and other neurological diseases (278, 279). These effects may be due to the fact that ketosis (due to caloric restriction/intermittent fasting, ketogenic diet) increase neurotrophic factors such as BDNF, increases levels of antioxidants, and reduces pro-inflammatory cytokine production (280, 282).

Both fasting and consumption of a ketogenic diet (55–60% fat, 30–35% protein, 5–10% carbohydrate) result in the production of ketone bodies (283). Two metabolic processes are critical in producing energy: gluconeogenesis and ketogenesis. Gluconeogenesis is the endogenous production of glucose in the body primarily from lactic acid, glycerol, and the amino acids alanine and glutamine. When glucose levels are low for prolonged periods (as with fasting), the endogenous production of glucose is not able to keep up with the needs of the body and ketogenesis is primarily used to derive energy (8, 278). Fatty acids and some amino acids are metabolized to form basic ketone bodies which accumulate in the body including: acetoacetate, beta-hydroxybutyrate (BHB), and acetone (8, 278). Ketone bodies may play an important role in mediating the beneficial effects of intermittent fasting and the ketogenic diet on the brain (276).

Ketone bodies are beneficial in humans with PD and animal models of PD. One early study found beneficial effects of hyperketonemia on PD patients (284). Likewise, in a rodent model of PD, BHB is associated with protection against MPTP-induced damage to dopaminergic neurons (285). Furthermore, BHB injection into the brain can rescue mitochondrial function and ameliorate dopaminergic neurodegeneration and motor deficits induced by MPTP in mice (286).

The effects of ketone bodies may be the consequence of a wide variety of mechanisms. For example, ketone bodies can cross the blood brain barrier and may bypass the type 1 complex mitochondrial defect in PD to rescue mitochondrial ATP function (8, 278). Another intriguing potential mechanism is the effects of ketone bodies on the NLRP3 inflammasome (287). For example, fasting can inhibit NLRP3 activation, which is thought to be due to effects of BHB (288, 289). Indeed, BHB directly inhibits the NLRP3 inflammasome and attenuates NLRP3-mediated inflammatory disease (287, 290). Likewise, fasting MPTP mice decreases IL-1β, a marker for NLRP3 activation (218).

In addition to ketone bodies, fasting and consumption of a ketogenic diet can also impact PD pathogenesis by influencing intestinal peptide production (i.e., GLP-1 and GIP) with downstream effects on NLRP3 inflammasome, insulin resistance, and BDNF production (276). Indeed, caloric restriction increases brain BDNF in a primate model of PD (282). Recent studies in MPTP mice shows that fasting increases BDNF in the brain (276).

Also, it appears that fasting impacts normal insulin signaling. Every other day fasting also corrects insulin resistance/T2DM in mice (291). This affect appears to be specific to changes in the intestinal microbiome, including the production of SCFA. Transfer of stool from mice fed every other day into mice with T2DM was sufficient to improve insulin resistance in the recipient mice similar to that observed due to every other day fasting itself (291). Thus, microbiota SCFA, IGN, and/or GLP-1 mediated mechanisms discussed above may play a role in the fasting effects as well. Intermittent fasting also promotes secondary bile acid production and improves intestinal barrier function in mice by restructuring the microbiome to produce more SCFA (292). Finally, ghrelin is another intestinal peptide that is produced in response to fasting and ghrelin is neuroprotective in the PD MPTP model (293). It is thought that the ghrelin protective mechanism may be by

promoting mitochondrial health and preventing NLRP3 IL-1β production (293–295).

Collectively, there is evidence that fasting and a ketogenic diet might be beneficial in PD and this effect may be mediated in significant part by changes in the intestinal microbiota. However, once again a well-designed trial is needed to show if the ketogenic diet is beneficial in PD before any serious consideration of fasting/ketogenic diet in the clinical care of PD patients.

OMEGA 3 POLYUNSATURATED FATTY ACIDS

Consumption of PUFAs is also an element of the Mediterranean diet and generally protective against neurodegeneration in AD or PD (296). There are three principal types of omega-3 (ω3) PUFAs including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA, typically from fish oil), and alpha-linolenic acid (ALA) (296). Dietary supplementation with PUFAs reduces depression in PD patients, which is important because depressive symptoms are common in PD patients and often impact other clinical aspects of the disease (297). In addition, EPA are neuroprotective in several neurodegenerative diseases including PD (6, 18, 298–300). Rodent models of PD also show benefit of PUFA administration. Consumption of an EPA-enriched diet lessens MPTP-induced movement dysfunction (i.e., hypokinesia) and ameliorates memory deficits in mice (298, 301). Administration of DHA reduces 6-OHDA-induced behavior deficits (i.e., ipsilateral rotations) and increases tyrosine hydroxylase (the enzyme required to produce dopamine) levels in a PD rat model (302). Experimentally, DHA is often combined with uridine monophosphate (UMP, a dietary precursor for membrane phospholipid synthesis), the DHA/UMP combination prevents the development of PD-like behavior and pathology in oral and striatal administration of rotenone models (61, 303). In addition, DHA/UMP combination reduces parkinsonian-like behaviors and elevates dopamine levels in 6-OHDA treated rodents (302). There are many mechanisms by which ω3 fatty acids may impact the brain and be beneficial in the prevention and/or treatment of PD. *GLP-1 stimulation*: As noted above, fish oil and olive oil can stimulate GLP-1/GIP production by the intestine (241). *Cell Death*: Studies have revealed that supplementation with EPA or DHA attenuates dopaminergic cell death induced by MPTP administration (301, 304). DHA may protect neurons against cytotoxicity through a variety of mechanisms such as inhibition of nitric oxide production, inhibition of caspase signaling pathways (305), inhibition of tau hyperphosphorylation (306), as well as regulation of other signaling pathways (e.g., PI3K/Akt). *Cell Function*: In addition to inhibiting neuronal cell death, DHA promotes optimal dopaminergic structure and function including synaptic plasticity (synapse formation, dendritic spine density) and dopaminergic neurotransmission (303, 307). *Inflammation*: The protective effects of DHA may be mediated by a metabolic derivative known as neuroprotectin D1 (NPD1) (308, 309) which is an inhibitor of NLRP3 (310). Indeed, NPD1 protects neurons against oxidative stress, inflammation, and from activation of apoptotic signaling pathways. Thus, while Western diet saturated fats activate the NLRP3 inflammasome (11), consumption of ω3 fatty acids inhibit the NLRP3 inflammasome (including in brain microglia) probably via a mechanism involving reduced mitochondrial stress (311–313). It should not be surprising then that ω3 fatty acids prevent NLRP3 inflammasome-dependent inflammation and insulin resistance in a T2DM rodent model (314). *Other*: DHA may also protect the brain by increasing glutathione reductase activity essentially preventing protein oxidation (315, 316), lipid peroxidation, and the production of ROS (317). Other potential mechanisms of action of DHA include regulation of NF-κB activation, transcription modulation, and cell membrane properties (318, 319). Again, these data provide a strong scientific rationale for conducting randomized controlled dietary trials in PD to determine whether PUFA supplements can impact neuroinflammation and the disease course of PD patients before recommending it to PD patients.

CONCLUSION

There is a growing body of experimental *in vitro*, *in vivo* animal and epidemiological evidence strongly suggesting that diet impacts the development/progression of multiple neurodegenerative diseases including PD. This includes both beneficial effects of diets rich in fiber, bioflavonoids, and ω3 fatty acids (e.g., the Mediterranean diet), and fasting and the ketogenic diet due the production of ketone bodies as well as the collective detrimental effects of the Western diet that include gut leakiness, NLRP3 activation, insulin resistance, and lack of beneficial SCFA/GLP-1 vagal signaling due to low fiber content. As we have discussed many of these effects may be due in large part to beneficial or negative effects on the intestinal microbiota. Diet rapidly and robustly alters the intestinal microbiome; thus, it is possible that these effects of diet are mediated (at least in part) by changes in microbiota structure and or function.

We described a mechanism by which intestinal dysbiosis can trigger intestinal barrier dysfunction leading to gut-derived LPS with systemic and neuroinflammation. We also described how bacterial components such as LPS can serve as a first signal in NLRP3 inflammasome mediated production of IL-1β, insulin resistance, and mitochondrial dysfunction. Finally, we described how bacterial metabolites such as SCFA and secondary bile acids can directly improve mitochondrial health as well as influence the production of the intestinal peptides GLP-1 and GIP that can directly promote brain health and stimulate IGN and together also regulate vagal stimulation of BDNF in the brain as well.

These data suggest that consumption of a Mediterranean diet might be a useful approach to prevent and possibly treat PD. This is because the characteristic features of the Mediterranean diet including high dietary fiber, bioflavonoids, and ω3 fatty acids that will modulate the microbiome and intestinal cell signaling and result in several alterations that confer benefits in the brain such as improved intestinal and blood brain barrier function, decreased NLRP3 inflammasome activation and IL-1β

production, improved insulin sensitivity, increased GLP-1/GIP, IGN vagal stimulation, and increased production of BDNF in the brain. Even if not adhering to the Mediterranean diet, including dietary supplements for dietary fiber, bioflavonoids, or ω3 fatty acids may be beneficial. Similar benefits may be obtained by following a diet involving intermittent fasting or a ketogenic diet.

Further investigations into the mechanisms by which the intestinal microbiota contributes to the development and progression of PD are warranted. More importantly, there is a major unmet need to determine whether dietary intervention can prevent progression of PD from the prodromal phase to the overt CNS/motor phase and whether dietary intervention can modify disease course and disease progression (and response to levodopa treatment) in those who suffer from motor symptoms. We believe that the experimental data and epidemiological findings discussed above provided a strong scientific rationale to conduct well-designed dietary and intestinal microbiota-directed randomized control trials (RCT) in both prodromal and established PD patents.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the writing of this review. AJ, CF, AK, RV, PE, and VR wrote the first draft. MS edited the first draft and the final version. All authors edited and approved the final draft. All authors edited and contributed to the final revised resubmission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.01245/full#supplementary-material

REFERENCES

1. Lees AJ, Hardy J, Revesz T. Parkinson's disease. *Lancet*. (2009) 373:2055–66. doi: 10.1016/S0140-6736(09)60492-X
2. Kalia LV, Lang AE. Parkinson's disease. *Lancet*. (2015) 386:896–912. doi: 10.1016/S0140-6736(14)61393-3
3. Obeso JA, Stamelou M, Goetz CG, Poewe W, Lang AE, Weintraub D, et al. Past, present, future of Parkinson's disease: a special essay on the 200th Anniversary of the Shaking Palsy. *Mov Disord*. (2017) 32:1264–310. doi: 10.1002/mds.26904
4. Marras C, Canning CG, Goldman SM. Environment, lifestyle, Parkinson's disease: Implications for prevention in the next decade. *Mov Disord*. (2019) 34:801–11. doi: 10.1002/mds.27720
5. Seidl SE, Santiago JA, Bilyk H, Potashkin JA. The emerging role of nutrition in Parkinson's disease. *Front Aging Neurosci*. (2014) 6:36. doi: 10.3389/fnagi.2014.00036
6. Perez-Pardo P, Kliest T, Dodiya HB, Broersen LM, Garssen J, Keshavarzian A, et al.Perez-Pardo P, et al. The gut-brain axis in Parkinson's disease: Possibilities for food-based therapies. *Eur J Pharmacol*. (2017) 817:86–95. doi: 10.1016/j.ejphar.2017.05.042
7. Rassmussen H, Piazza BR, Forsyth CB, Keshavarzian A. Nutrition and gastrointestinal health as modulators of Parkinson's disease. In: In: Folkerts G, Garssen J, editors. *Pharma-Nutrition*. AAPS Advances in the Pharmaceutical Sciences Series. New York, NY: Springer International Publishing (2014). p. 213–42. doi: 10.1007/978-3-319-06151-1_11
8. Mattson MP. Interventions that improve body and brain bioenergetics for Parkinson's disease risk reduction and therapy. *J Parkinsons Dis*. (2014) 4:1–13. doi: 10.3233/JPD-130335
9. Mischley LK, Lau RC, Bennett RD. Role of diet and nutritional supplements in Parkinson's disease progression. *Oxid Med Cell Longev*. (2017) 2017:6405278. doi: 10.1155/2017/6405278
10. Makki K, Deehan EC, Walter J, Bäckhed F. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe*. (2018) 23:705–15. doi: 10.1016/j.chom.2018.05.012
11. Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, et al. Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell*. (2018) 172:162–75.e14. doi: 10.1016/j.cell.2017.12.013
12. Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutr J*. (2014) 13:61. doi: 10.1186/1475-2891-13-61
13. Barichella M, Cereda E, Cassani E, Pinelli G, Iorio L, Ferri V, et al. Dietary habits and neurological features of Parkinson's disease patients: implications for practice. *Clin Nutr*. (2017) 36:1054–61. doi: 10.1016/j.clnu.2016.06.020
14. Liddle RA. Parkinson's disease from the gut. *Brain Res*. (2018) 1693(Pt B):201–6. doi: 10.1016/j.brainres.2018.01.010
15. Anderson C, Checkoway H, Franklin GM, Beresford S, Smith-Weller T, Swanson PD. Dietary factors in Parkinson's disease: the role of food groups and specific foods. *Mov Disord*. (1999) 14:21–7.
16. Maraki MI, Yannakoulia M, Stamelou M, Stefanis L, Xiromerisiou G, Kosmidis MH, et al. Mediterranean diet adherence is related to reduced probability of prodromal Parkinson's disease. *Mov Disord*. (2019) 34:48–57. doi: 10.1002/mds.27489
17. Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A. Habitual intake of dietary flavonoids and risk of Parkinson disease. *Neurology*. (2012) 78:1138–45. doi: 10.1212/WNL.0b013e31824f7fc4
18. Bousquet M, Calon F, Cicchetti F, Impact of omega-3 fatty acids in Parkinson's disease. *Ageing Res Rev*. (2011) 10:453–63. doi: 10.1016/j.arr.2011.03.001
19. Kamel F, Goldman SM, Umbach DM, Chen H, Richardson G, Barber MR, et al. Dietary fat intake, pesticide use, Parkinson's disease. *Parkinsonism Relat Disord*. (2013) 20:82–7. doi: 10.1016/j.parkreldis.2013.09.023
20. Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, implications for health and disease. *Gastroenterology*. (2014) 146:1564–72. doi: 10.1053/j.gastro.2014.01.058
21. Heiman ML, Greenway FL. A healthy gastrointestinal microbiome is dependent on dietary diversity. *Mol Metab*. (2016) 5:317–20. doi: 10.1016/j.molmet.2016.02.005
22. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. (2014) 505:559–63. doi: 10.1038/nature12820
23. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*. (2012) 489:242–9. doi: 10.1038/nature11552
24. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med*. (2018) 24:392–400. doi: 10.1038/nm.4517
25. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science*. (2011) 334:105–8. doi: 10.1126/science.1208344
26. Ross GW, Abbott RD, Petrovitch H, Morens DM, Grandinetti A, Tung KH, et al. Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA*. (2000) 283:2674–9. doi: 10.1001/jama.283.20.2674

27. Hernán MA, Takkouche B, Caamaño-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, the risk of Parkinson's disease. *Ann Neurol.* (2002) 52:276–84. doi: 10.1002/ana.10277
28. Tan EK, Tan C, Fook-Chong SM, Lum SY, Chai A, Chung H, et al. Dose-dependent protective effect of coffee, tea, smoking in Parkinson's disease: a study in ethnic Chinese. *J Neurol Sci.* (2003) 216:163–7. doi: 10.1016/j.jns.2003.07.006
29. Kim IY, O'Reilly ÉJ, Hughes KC, Gao X, Schwarzschild MA, Ascherio A. Differences in Parkinson's disease risk with caffeine intake and postmenopausal hormone use. *J Parkinsons Dis.* (2017) 7:677–84. doi: 10.3233/JPD-171175
30. Palacios N, Gao X, McCullough ML, Schwarzschild MA, Shah R, Gapstur S, et al. Caffeine and risk of Parkinson's disease in a large cohort of men and women. *Mov Disord.* (2012) 27:1276–82. doi: 10.1002/mds.25076
31. Maggio R, Riva M, Vaglini F, Fornai F, Molteni R, Armogida M, et al. Nicotine prevents experimental parkinsonism in rodents and induces striatal increase of neurotrophic factors. *J Neurochem.* (1998) 71:2439–46. doi: 10.1046/j.1471-4159.1998.71062439.x
32. Chen JF, Xu K, Petzer JP, Staal R, Xu YH, Beilstein M, et al. Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. *J Neurosci.* (2001) 21:RC143. doi: 10.1523/JNEUROSCI.21-10-j0001.2001
33. Shi A, Shi H, Wang Y, Liu X, Cheng Y, Li H, et al. Activation of Nrf2 pathway and inhibition of NLRP3 inflammasome activation contribute to the protective effect of chlorogenic acid on acute liver injury. *Int Immunopharmacol.* (2018) 54:125–30. doi: 10.1016/j.intimp.2017.11.007
34. Yan R, Zhang J, Park HJ, Park ES, Oh S, Zheng H, et al. Synergistic neuroprotection by coffee components eicosanoyl-5-hydroxytryptamide and caffeine in models of Parkinson's disease and DLB. *Proc Natl Acad Sci USA.* (2018) 115:E12053–62. doi: 10.1073/pnas.1813365115
35. Kujawska M, Jodynis-Liebert J. Polyphenols in Parkinson's disease: a systematic review of *in vivo* studies. *Nutrients*. (2018) 10:E642. doi: 10.3390/nu10050642
36. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, et al. Impact of dietary polyphenols on carbohydrate metabolism. *Int J Mol Sci.* (2010) 11:1365–402. doi: 10.3390/ijms11041365
37. Derkinderen P, Shannon KM, Brundin P. Gut feelings about smoking and coffee in Parkinson's disease. *Mov Disord.* (2014) 29:976–9. doi: 10.1002/mds.25882
38. Scheperjans F, Pekkonen E, Kaakkola S, Auvinen P. Linking smoking, coffee, urate, Parkinson's disease - A role for gut microbiota? *J Parkinsons Dis.* (2015) 5:255–62. doi: 10.3233/JPD-150557
39. Palacios N, Gao X, O'Reilly E, Schwarzschild M, McCullough ML, Mayo T, et al. Alcohol and risk of Parkinson's disease in a large, prospective cohort of men and women. *Mov Disord.* (2012) 27:980–7. doi: 10.1002/mds.25050
40. Hernán MA, Chen H, Schwarzschild MA, Ascherio A. Alcohol consumption and the incidence of Parkinson's disease. *Ann Neurol.* (2003) 54:170–5. doi: 10.1002/ana.10611
41. Ishihara L, Brayne C. A systematic review of nutritional risk factors of Parkinson's disease. *Nutr Res Rev.* (2005) 18:259–82. doi: 10.1079/NRR2005108
42. Brighina L, Schneider NK, Lesnick TG, de Andrade M, Cunningham JM, Mrazek D, et al.Brighina L, et al. Alpha-synuclein, alcohol use disorders, Parkinson disease: a case-control study. Parkinsonism Relat Disord. (2009) 15:430–4. doi: 10.1016/j.parkreldis.2008.11.011
43. Bettiol SS, Rose TC, Hughes CJ, Smith LA. Alcohol consumption and Parkinson's disease risk: A review of recent findings. *J Parkinsons Dis.* (2015) 5:425–42. doi: 10.3233/JPD-150533
44. Hillemacher T, Bachmann O, Kahl KG, Frieling H. Alcohol, microbiome, their effect on psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* (2018) 85:105–15. doi: 10.1016/j.pnpbp.2018.04.015
45. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol.* (2012) 302:G966–78. doi: 10.1152/ajpgi.00380.2011
46. Engen PA, Green SJ, Voigt RM, Forsyth CB, Keshavarzian A. The gastrointestinal microbiome: alcohol effects on the composition of intestinal microbiota. *Alcohol Res.* (2015) 37:223–36.
47. Chen H, O'Reilly E, McCullough ML, Rodriguez C, Schwarzschild MA, Calle EE, et al. Consumption of dairy products and risk of Parkinson's disease. *Am J Epidemiol.* (2007) 165:998–1006. doi: 10.1093/aje/kwk089
48. Chen H, Zhang SM, Hernán MA, Willett WC, Ascherio A. Diet and Parkinson's disease: a potential role of dairy products in men. *Ann Neurol.* (2002) 52:793–801. doi: 10.1002/ana.10381
49. Park M, Ross GW, Petrovitch H, White LR, Masaki KH, Nelson JS, et al. Consumption of milk and calcium in midlife and the future risk of Parkinson disease. *Neurology.* (2005) 64:1047–51. doi: 10.1212/01.WNL.0000154532.98495.BF
50. Kyrozis A, Ghika A, Stathopoulos P, Vassilopoulos D, Trichopoulos D, Trichopoulou A. Dietary and lifestyle variables in relation to incidence of Parkinson's disease in Greece. *Eur J Epidemiol.* (2013) 28:67–77. doi: 10.1007/s10654-012-9760-0
51. Hughes KC, Gao X, Kim IY, Wang M, Weisskopf MG, Schwarzschild MA, et al. Intake of dairy foods and risk of Parkinson disease. *Neurology.* (2017) 89:46–52. doi: 10.1212/WNL.0000000000004057
52. Jiang W, Ju C, Jiang H, Zhang D. Dairy foods intake and risk of Parkinson's disease: a dose-response meta-analysis of prospective cohort studies. *Eur J Epidemiol.* (2014) 29:613–9. doi: 10.1007/s10654-014-9921-4
53. Abbott RD, Ross GW, Petrovitch H, Masaki KH, Launer LJ, Nelson JS, et al. Midlife milk consumption and substantia nigra neuron density at death. *Neurology.* (2016) 86:512–9. doi: 10.1212/WNL.0000000000002254
54. Tetz G, Brown SM, Hao Y, Tetz V. Parkinson's disease and bacteriophages as its overlooked contributors. *Sci Rep.* (2018) 8:10812. doi: 10.1038/s41598-018-29173-4
55. Kistner A, Krack P. Parkinson's disease: no milk today? *Front Neurol.* (2014) 5:172. doi: 10.3389/fneur.2014.00172
56. Hatcher JM, Pennell KD, Miller GW. Parkinson's disease and pesticides: a toxicological perspective. *Trends Pharmacol Sci.* (2008) 29:322–9. doi: 10.1016/j.tips.2008.03.007
57. Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. Rotenone, paraquat, Parkinson's disease. *Environ Health Perspect.* (2011) 119:866–72. doi: 10.1289/ehp.1002839
58. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem.* (1990) 54:823–7. doi: 10.1111/j.1471-4159.1990.tb02325.x
59. Zhu J, Chu CT, Mitochondrial dysfunction in Parkinson's disease. *J Alzheimers Dis.* (2010) 20(Suppl. 2):S325–34. doi: 10.3233/JAD-2010-100363
60. Salminen A, Ojala J, Kaarniranta K, Kauppinen A. Mitochondrial dysfunction and oxidative stress activate inflammasomes: impact on the aging process and age-related diseases. *Cell Mol Life Sci.* (2012) 69:2999–3013. doi: 10.1007/s00018-012-0962-0
61. Perez-Pardo P, Dodiya HB, Broersen LM, Douna H, van Wijk N, Lopes da Silva S, et al. Gut-brain and brain-gut axis in Parkinson's disease models: effects of a uridine and fish oil diet. *Nutr Neurosci.* (2017) 21:391–402. doi: 10.1080/1028415X.2017.1294555
62. Greenamyre JT, Cannon JR, Drolet R, Mastroberardino PG. Lessons from the rotenone model of Parkinson's disease. *Trends Pharmacol Sci.* (2010) 31:141–2; author reply:142–3. doi: 10.1016/j.tips.2009.12.006
63. Langston JW. The MPTP story. *J Parkinsons Dis.* (2017) 7:S11–9. doi: 10.3233/JPD-179006
64. Cicchetti F, Drouin-Ouellet J, Gross RE. Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? *Trends Pharmacol Sci.* (2009) 30:475–83. doi: 10.1016/j.tips.2009.06.005
65. Priyadarshi A, Khuder SA, Schaub EA, Shrivastava S. A meta-analysis of Parkinson's disease and exposure to pesticides. *Neurotoxicology.* (2000) 21:435–40.
66. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science.* (1983) 219:979–80. doi: 10.1126/science.6823561
67. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, et al. Pesticide exposure and risk for Parkinson's disease. *Ann Neurol.* (2006) 60:197–203. doi: 10.1002/ana.20904
68. Perez-Pardo P, Dodiya HB, Engen PA, Forsyth CB, Huschens AM, Shaikh M, et al. Role of TLR4 in the gut-brain axis in Parkinson's

disease: a translational study from men to mice. *Gut.* (2018) 68:829–43. doi: 10.1136/gutjnl-2018-316844
69. Perez-Pardo P, Dodiya HB, Engen PA, Naqib A, Forsyth CB, Green SJ, et al. Gut bacterial composition in a mouse model of Parkinson's disease. *Benef Microbes.* (2018) 9:799–814. doi: 10.3920/BM2017.0202
70. Johnson ME, Stringer A, Bobrovskaya L. Rotenone induces gastrointestinal pathology and microbiota alterations in a rat model of Parkinson's disease. *Neurotoxicology.* (2018) 65:174–85. doi: 10.1016/j.neuro.2018.02.013
71. Gao B, Bian X, Chi L, Tu P, Ru H, Lu K. Editor's highlight: organophosphatediazinon altered quorum sensing, cell motility, stress response, carbohydrate metabolism of gut microbiome. *Toxicol Sci.* (2017) 157:354–64. doi: 10.1093/toxsci/kfx053
72. Killinger BA, Labrie V. Vertebrate food products as a potential source of prion-like alpha-synuclein. *NPJ Parkinsons Dis.* (2017) 3:33. doi: 10.1038/s41531-017-0035-z
73. Uversky VN, Eliezer D. Biophysics of Parkinson's disease: structure and aggregation of alpha-synuclein. *Curr Protein Pept Sci.* (2009) 10:483–99. doi: 10.2174/138920309789351921
74. Stefanis L. alpha-Synuclein in Parkinson's disease. *Cold Spring Harb Perspect Med.* (2012) 2:a009399. doi: 10.1101/cshperspect.a009399
75. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. *J Exp Med.* (2012) 209:975–86. doi: 10.1084/jem.20112457
76. Luk KC, Song C, O'Brien P, Stieber A, Branch JR, Brunden KR, et al. Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *Proc Natl Acad Sci USA.* (2009) 106:20051–6. doi: 10.1073/pnas.0908005106
77. Gordon R, Albornoz EA, Christie DC, Langley MR, Kumar V, Mantovani S, et al. Inflammasome inhibition prevents alpha-synuclein pathology and dopaminergic neurodegeneration in mice. *Sci Transl Med.* (2018) 10:eaah4066. doi: 10.1126/scitranslmed.aah4066
78. Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, et al. alpha-Synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature.* (2015) 522:340–4. doi: 10.1038/nature14547
79. Rota L, Pellegrini C, Benvenuti L, Antonioli L, Fornai M, Blandizzi C, et al. Constipation, deficit in colon contractions and alpha-synuclein inclusions within the colon precede motor abnormalities and neurodegeneration in the central nervous system in a mouse model of alpha-synucleinopathy. *Transl Neurodegener.* (2019) 8:5. doi: 10.1186/s40035-019-0146-z
80. Kim S, Kwon SH, Kam TI, Panicker N, Karuppagounder SS, Lee S, et al. Transneuronal propagation of pathologic alpha-synuclein from the gut to the brain models Parkinson's disease. *Neuron.* (2019) 103:627–41.e7. doi: 10.1016/j.neuron.2019.05.035
81. Holmqvist S, Chutna O, Bousset L, Aldrin-Kirk P, Li W, Björklund T, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* (2014) 128:805–20. doi: 10.1007/s00401-014-1343-6
82. Donaldson DS, Kobayashi A, Ohno H, Yagita H, Williams IR, Mabbott NA. M cell-depletion blocks oral prion disease pathogenesis. *Mucosal Immunol.* (2012) 5:216–25. doi: 10.1038/mi.2011.68
83. Wood H. Parkinson disease: LAG3 facilitates cell-to-cell spread of alpha-synuclein pathology. *Nat Rev Neurol.* (2016) 12:678. doi: 10.1038/nrneurol.2016.164
84. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE.* (2011) 6:e28032. doi: 10.1371/journal.pone.0028032
85. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature.* (2012) 486:207–14. doi: 10.1038/nature11234
86. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* (2010) 464:59–65. doi: 10.1038/nature08821
87. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell.* (2016) 165:1332–45. doi: 10.1016/j.cell.2016.05.041
88. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the gastrointestinal microbiome with nondigestible fermentable carbohydrates to improve human health. *Microbiol Spectr.* (2017) 5:1–24. doi: 10.1128/microbiolspec.BAD-0019-2017
89. Garcia-Mantrana I, Selma-Royo M, Alcantara C, Collado MC. Shifts on gut microbiota associated to mediterranean diet adherence and specific dietary intakes on general adult population. *Front Microbiol.* (2018) 9:890. doi: 10.3389/fmicb.2018.00890
90. Gilbert JA, Quinn RA, Debelius J, Xu ZZ, Morton J, Garg N, et al. Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature.* (2016) 535:94–103. doi: 10.1038/nature18850
91. Buford TW. (Dis)Trust your gut: the gut microbiome in age-related inflammation, health, disease. *Microbiome.* (2017) 5:80. doi: 10.1186/s40168-017-0296-0
92. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell.* (2012) 148:1258–70. doi: 10.1016/j.cell.2012.01.035
93. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. *Nat Rev Genet.* (2012) 13:260–70. doi: 10.1038/nrg3182
94. Bell JS, Spencer JI, Yates RL, Yee SA, Jacobs BM, DeLuca GC. Invited review: from nose to gut - the role of the microbiome in neurological disease. *Neuropathol Appl Neurobiol.* (2019) 45:195–215. doi: 10.1111/nan.12520
95. Lubomski M, Tan AH, Lim SY, Holmes AJ, Davis RL, Sue CM. Parkinson's disease and the gastrointestinal microbiome. *J Neurol.* (2019) 91:1–17. doi: 10.1007/s00415-019-09320-1
96. Sampson T. The impact of indigenous microbes on Parkinson's disease. *Neurobiol Dis.* (2019) 104426. doi: 10.1016/j.nbd.2019.03.014
97. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord.* (2014) 30:350–8. doi: 10.1002/mds.26069
98. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord.* (2015) 30:1351–60. doi: 10.1002/mds.26307
99. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature.* (2012) 489:220–30. doi: 10.1038/nature11550
100. Caporaso JG, Lauber CL, Costello EK, Berg-Lyons D, Gonzalez A, Stombaugh J, et al. Moving pictures of the human microbiome. *Genome Biol.* (2011) 12:R50. doi: 10.1186/gb-2011-12-5-r50
101. Noble EE, Hsu TM, Kanoski SE. Gut to brain dysbiosis: mechanisms linking western diet consumption, the microbiome, cognitive impairment. *Front Behav Neurosci.* (2017) 11:9. doi: 10.3389/fnbeh.2017.00009
102. Miller GE, Engen PA, Gillevet PM, Shaikh M, Sikaroodi M, Forsyth CB, et al. Lower neighborhood socioeconomic status associated with reduced diversity of the colonic microbiota in healthy adults. *PLoS ONE.* (2016) 11:e0148952. doi: 10.1371/journal.pone.0148952
103. Aho VTE, Pereira PAB, Voutilainen S, Paulin L, Pekkonen E, Auvinen P, et al. Gut microbiota in Parkinson's disease: temporal stability and relations to disease progression. *EBioMedicine.* (2019) 44:691–707. doi: 10.1016/j.ebiom.2019.05.064
104. Minato T, Maeda T, Fujisawa Y, Tsuji H, Nomoto K, Ohno K, et al. Progression of Parkinson's disease is associated with gut dysbiosis: Two-year follow-up study. *PLoS ONE.* (2017) 12:e0187307. doi: 10.1371/journal.pone.0187307
105. Heintz-Buschart A, Pandey U, Wicke T, Sixel-Döring F, Janzen A, Sittig-Wiegand E, et al. The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. *Mov Disord.* (2018) 33:88–98. doi: 10.1002/mds.27105
106. Mihaila D, Donegan J, Barns S, LaRocca D, Du Q, Zheng D, et al. The oral microbiome of early stage Parkinson's disease and its relationship with functional measures of motor and non-motor function. *PLoS ONE.* (2019) 14:e0218252. doi: 10.1371/journal.pone.0218252
107. Pereira PAB, Aho VTE, Paulin L, Pekkonen E, Auvinen P, Scheperjans F. Oral and nasal microbiota in Parkinson's disease. *Parkinsonism Relat Disord.* (2017) 38:61–7. doi: 10.1016/j.parkreldis.2017.02.026
108. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Bürmann J, et al. Short chain fatty acids and gut microbiota differ between patients with

Parkinson's disease and age-matched controls. *Parkinsonism Relat Disord.* (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
109. Bedarf JR, Hildebrand F, Coelho LP, Sunagawa S, Bahram M, Goeser F, et al. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naive Parkinson's disease patients. *Genome Med.* (2017) 9:39. doi: 10.1186/s13073-017-0451-z
110. Hill-Burns EM, Debelius JW, Morton JT, Wissemann WT, Lewis MR, Wallen ZD, et al. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Mov Disord.* (2017) 32:739–49. doi: 10.1002/mds.26942
111. Barichella M, Severgnini M, Cilia R, Cassani E, Bolliri C, Caronni S, et al. Unraveling gut microbiota in Parkinson's disease and atypical Parkinsonism. *Mov Disord.* (2019) 34:396–405. doi: 10.1002/mds.27581
112. Li C, Cui L, Yang Y, Miao J, Zhao X, Zhang J, et al. Gut microbiota differs between Parkinson's disease patients and healthy controls in northeast China. *Front Mol Neurosci.* (2019) 12:171. doi: 10.3389/fnmol.2019.00171
113. Petrov VA, Saltykova IV, Zhukova IA, Alifirova VM, Zhukova NG, Dorofeeva YB, et al. Analysis of gut microbiota in patients with Parkinson's disease. *Bull Exp Biol Med.* (2017) 162:734–7. doi: 10.1007/s10517-017-3700-7
114. Lin A, Zheng W, He Y, Tang W, Wei X, He R, et al. Gut microbiota in patients with Parkinson's disease in southern China. *Parkinsonism Relat Disord.* (2018) 53:82–8. doi: 10.1016/j.parkreldis.2018.05.007
115. Hasegawa S, Goto S, Tsuji H, Okuno T, Asahara T, Nomoto K, et al. Intestinal dysbiosis and lowered serum lipopolysaccharide-binding protein in Parkinson's disease. *PLoS ONE.* (2015) 10:e0142164. doi: 10.1371/journal.pone.0142164
116. Hopfner F, Künstner A, Müller SH, Künzel S, Zeuner KE, Margraf NG, et al. Gut microbiota in Parkinson disease in a northern German cohort. *Brain Res.* (2017) 1667:41–5. doi: 10.1016/j.brainres.2017.04.019
117. Li W, Wu X, Hu X, Wang T, Liang S, Duan Y, et al. Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. *Sci China Life Sci.* (2017) 60:1223–33. doi: 10.1007/s11427-016-9001-4
118. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol.* (2013) 31:814–21. doi: 10.1038/nbt.2676
119. Qian Y, Yang X, Xu S, Wu C, Song Y, Qin N, et al. Alteration of the fecal microbiota in Chinese patients with Parkinson's disease. *Brain Behav Immun.* (2018) 70:194–202. doi: 10.1016/j.bbi.2018.02.016
120. Braak H, Rüb U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm.* (2003) 110:517–36. doi: 10.1007/s00702-002-0808-2
121. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* (2007) 33:599–614. doi: 10.1111/j.1365-2990.2007.00874.x
122. Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Parkinsonism Relat Disord.* (2011) 17:10–5. doi: 10.1016/j.parkreldis.2010.08.003
123. Fasano A, Visanji NP, Liu LW, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol.* (2015) 14:625–39. doi: 10.1016/S1474-4422(15)00007-1
124. Cassani E, Barichella M, Ferri V, Pinelli G, Iorio L, Bolliri C, et al. Dietary habits in Parkinson's disease: adherence to Mediterranean diet. *Parkinsonism Relat Disord.* (2017) 42:40–6. doi: 10.1016/j.parkreldis.2017.06.007
125. Zhou ZL, Jia XB, Sun MF, Zhu YL, Qiao CM, Zhang BP, et al. Neuroprotection of fasting mimicking diet on MPTP-induced Parkinson's disease mice via gut microbiota and metabolites. *Neurotherapeutics.* (2019) 16:741–60. doi: 10.1007/s13311-019-00719-2
126. Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, et al. Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology.* (2001) 57:456–62. doi: 10.1212/WNL.57.3.456
127. Knudsen K, Krogh K, Østergaard K, Borghammer P. Constipation in Parkinson's disease: subjective symptoms, objective markers, new perspectives. *Mov Disord.* (2017) 32:94–105. doi: 10.1002/mds.26866
128. Khalif IL, Quigley EM, Konovitch EA, Maximova ID. Alterations in the colonic flora and intestinal permeability and evidence of immune activation in chronic constipation. *Dig Liver Dis.* (2005) 37:838–49. doi: 10.1016/j.dld.2005.06.008
129. Videnovic A, Golombek D. Circadian and sleep disorders in Parkinson's disease. *Exp Neurol.* (2013) 243:45–56. doi: 10.1016/j.expneurol.2012.08.018
130. Videnovic A, Willis GL. Circadian system - A novel diagnostic and therapeutic target in Parkinson's disease? *Mov Disord.* (2016) 31:260–9. doi: 10.1002/mds.26509
131. Voigt RM, Forsyth CB, Green SJ, Engen PA, Keshavarzian A. Circadian rhythm and the gut microbiome. *Int Rev Neurobiol.* (2016) 131:193–205. doi: 10.1016/bs.irn.2016.07.002
132. Paschos GK, FitzGerald GA. Circadian clocks and metabolism: implications for microbiome and aging. *Trends Genet.* (2017) 33:760–9. doi: 10.1016/j.tig.2017.07.010
133. Scheperjans F, Derkinderen P, Borghammer P. The gut and Parkinson's disease: hype or hope? *J Parkinsons Dis.* (2018) 8:S31–9. doi: 10.3233/JPD-181477
134. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell.* (2016) 167:1469–80.e12. doi: 10.1016/j.cell.2016.11.018
135. Austin KW, Ameringer SW, Cloud LJ. An integrated review of psychological stress in Parkinson's disease: biological mechanisms and symptom and health outcomes. *Parkinsons Dis.* (2016) 2016:9869712. doi: 10.1155/2016/9869712
136. Chen H, Ritz B. The search for environmental causes of Parkinson's disease: moving forward. *J Parkinsons Dis.* (2018) 8:S9–17. doi: 10.3233/JPD-181493
137. Dodiya HB, Forsyth CB, Voigt RM, Engen PA, Patel J, Shaikh M, et al. Chronic stress-induced gut dysfunction exacerbates Parkinson's disease phenotype and pathology in a rotenone-induced mouse model of Parkinson's disease. *Neurobiol Dis.* (2018) 104352. doi: 10.1016/j.nbd.2018.12.012
138. Allen JM, Mailing LJ, Niemiro GM, Moore R, Cook MD, White BA, et al. Exercise alters gut microbiota composition and function in lean and obese humans. *Med Sci Sports Exerc.* (2018) 50:747–57. doi: 10.1249/MSS.0000000000001495
139. Borghammer P. How does Parkinson's disease begin? Perspectives on neuroanatomical pathways, prions, histology. *Mov Disord.* (2018) 33:48–57. doi: 10.1002/mds.27138
140. Lionnet A, Leclair-Visonneau L, Neunlist M, Murayama S, Takao M, Adler CH, et al. Does Parkinson's disease start in the gut? *Acta Neuropathol.* (2017) 135:1–12. doi: 10.1007/s00401-017-1777-8
141. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci USA.* (2009) 106:13010–5. doi: 10.1073/pnas.0903691106
142. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med.* (2008) 14:504–6. doi: 10.1038/nm1747
143. Kordower JH, Chu Y, Hauser RA, Olanow CW, Freeman TB. Transplanted dopaminergic neurons develop PD pathologic changes: a second case report. *Mov Disord.* (2008) 23:2303–6. doi: 10.1002/mds.22369
144. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med.* (2008) 14:501–3. doi: 10.1038/nm1746
145. Kujawska M, Jodynis-Liebert J. What is the evidence that Parkinson's disease is a prion disorder, which originates in the gut? *Int J Mol Sci.* (2018) 19:E3573. doi: 10.3390/ijms19113573
146. Riederer P, Berg D, Casadei N, Cheng F, Classen J, Dresel C, et al. α-synuclein in Parkinson's disease: causal or bystander? *J Neural Transm.* (2019) 126:815–40. doi: 10.1007/s00702-019-02025-9
147. Santos SF, de Oliveira HL, Yamada ES, Neves BC, Pereira A. The gut and Parkinson's disease-a bidirectional pathway. *Front Neurol.* (2019) 10:574. doi: 10.3389/fneur.2019.00574
148. Perez-Pardo P, Hartog M, Garssen J, Kraneveld AD. Microbes tickling your tummy: the importance of the gut-brain axis in Parkinson's Disease. *Curr Behav Neurosci Rep.* (2017) 4:361–8. doi: 10.1007/s40473-017-0129-2
149. Dutta G, Zhang P, Liu B. The lipopolysaccharide Parkinson's disease animal model: mechanistic studies and drug discovery. *Fundam Clin Pharmacol.* (2008) 22:453–64. doi: 10.1111/j.1472-8206.2008.00616.x

150. Zhao Y, Jaber V, Lukiw WJ. Secretory products of the human GI tract microbiome and their potential impact on Alzheimer's disease (AD): detection of lipopolysaccharide (LPS) in AD hippocampus. *Front Cell Infect Microbiol.* (2017) 7:318. doi: 10.3389/fcimb.2017.00318
151. Sampson TR, Mazmanian SK. Control of brain development, function, behavior by the microbiome. *Cell Host Microbe.* (2015) 17:565–76. doi: 10.1016/j.chom.2015.04.011
152. Bourassa MW, Alim I, Bultman SJ, Ratan RR. Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? *Neurosci Lett.* (2016) 625:56–63. doi: 10.1016/j.neulet.2016.02.009
153. Dinan TG, Cryan JF. Gut feelings on Parkinson's and depression. *Cerebrum.* (2017) 2017:cer-04-17.
154. Everard A, Cani PD. Gut microbiota and GLP-1. *Rev Endocr Metab Disord.* (2014) 15:189–96. doi: 10.1007/s11154-014-9288-6
155. Liu J, Wang F, Liu S, Du J, Hu X, Xiong J, et al. Sodium butyrate exerts protective effect against Parkinson's disease in mice via stimulation of glucagon like peptide-1. *J Neurol Sci.* (2017) 381:176–81. doi: 10.1016/j.jns.2017.08.3235
156. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. *Mov Disord.* (2012) 27:716–9. doi: 10.1002/mds.25020
157. Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. *Mov Disord.* (2012) 27:709–15. doi: 10.1002/mds.23838
158. Lebouvier T, Chaumette T, Paillusson S, Duyckaerts C, Bruley des Varannes S, Neunlist M, et al. The second brain and Parkinson' disease. *Eur J Neurosci.* (2009) 30:735–41. doi: 10.1111/j.1460-9568.2009.06873.x
159. Ruffmann C, Parkkinen L. Gut feelings about alpha-synuclein in gastrointestinal biopsies: biomarker in the making? *Mov Disord.* (2016) 31:193–202. doi: 10.1002/mds.26480
160. Stokholm MG, Danielsen EH, Hamilton-Dutoit SJ, Borghammer P. Pathological alpha-synuclein in gastrointestinal tissues from prodromal Parkinson disease patients. *Ann Neurol.* (2016) 79:940–9. doi: 10.1002/ana.24648
161. Knudsen K, Fedorova TD, Bekker AC, Iversen P, Østergaard K, Krogh K, et al. Objective colonic dysfunction is far more prevalent than subjective constipation in Parkinson's disease: a colon transit and volume study. *J Parkinsons Dis.* (2017) 7:359–67. doi: 10.3233/JPD-161050
162. Del Tredici K, Braak H. Review: sporadic Parkinson's disease: development and distribution of alpha-synuclein pathology. *Neuropathol Appl Neurobiol.* (2016) 42:33–50. doi: 10.1111/nan.12298
163. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, et al. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science.* (2012) 338:949–53. doi: 10.1126/science.1227157
164. Svensson E, Horváth-Puhó E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, et al. Vagotomy and subsequent risk of Parkinson's disease. *Ann Neurol.* (2015) 78:522–9. doi: 10.1002/ana.24448
165. Tysnes OB, Kenborg L, Herlofson K, Steding-Jessen M, Horn A, Olsen JH, et al. Does vagotomy reduce the risk of Parkinson's disease? *Ann Neurol.* (2015) 78:1011–2. doi: 10.1002/ana.24531
166. Liu B, Fang F, Pedersen NL, Tillander A, Ludvigsson JF, Ekbom A, et al. Vagotomy and Parkinson disease: a Swedish register-based matched-cohort study. *Neurology.* (2017) 88:1996–2002. doi: 10.1212/WNL.0000000000003961
167. Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. *J Physiol.* (2017) 595:489–503. doi: 10.1113/JP273106
168. Abdel-Haq R, Schlachetzki JCM, Glass CK, Mazmanian SK. Microbiome-microglia connections via the gut-brain axis. *J Exp Med.* (2018) 216:41–59. doi: 10.1084/jem.20180794
169. Mayer EA, Knight R, Mazmanian SK, Cryan JF, Tillisch K. Gut microbes and the brain: paradigm shift in neuroscience. *J Neurosci.* (2014) 34:15490–6. doi: 10.1523/JNEUROSCI.3299-14.2014
170. Sun MF, Zhu YL, Zhou ZL, Jia XB, Xu YD, Yang Q, et al. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: gut microbiota, glial reaction and TLR4/TNF-alpha signaling pathway. *Brain Behav Immun.* (2018) 70:48–60. doi: 10.1016/j.bbi.2018.02.005
171. Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol.* (2015) 21:10609–20. doi: 10.3748/wjg.v21.i37.10609
172. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci.* (2007) 8:57–69. doi: 10.1038/nrn2038
173. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell.* (2014) 156:84–96. doi: 10.1016/j.cell.2013.12.016
174. Athauda D, Foltynie T, The glucagon-like peptide 1 (GLP) receptor as a therapeutic target in Parkinson's disease: mechanisms of action. *Drug Discov Tdy.* (2016) 21:802–18. doi: 10.1016/j.drudis.2016.01.013
175. Obrenovich MEM. Leaky gut, leaky brain? *Microorganisms.* (2018) 6:107. doi: 10.3390/microorganisms6040107
176. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut.* (1987) 28:1221–7. doi: 10.1136/gut.28.10.1221
177. Ganapathy V, Thangaraju M, Prasad PD, Martin PM, Singh N. Transporters and receptors for short-chain fatty acids as the molecular link between colonic bacteria and the host. *Curr Opin Pharmacol.* (2013) 13:869–74. doi: 10.1016/j.coph.2013.08.006
178. Hong J, Jia Y, Pan S, Jia L, Li H, Han Z, et al. Butyrate alleviates high fat diet-induced obesity through activation of adiponectin-mediated pathway and stimulation of mitochondrial function in the skeletal muscle of mice. *Oncotarget.* (2016) 7:56071–82. doi: 10.18632/oncotarget.11267
179. Gruber J, Kennedy BK. Microbiome and longevity: gut microbes send signals to host mitochondria. *Cell.* (2017) 169:1168–9. doi: 10.1016/j.cell.2017.05.048
180. Tan J, McKenzie C, Vuillermin PJ, Goverse G, Vinuesa CG, Mebius RE, et al. Dietary fiber and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways. *Cell Rep.* (2016) 15:2809–24. doi: 10.1016/j.celrep.2016.05.047
181. Clairembault T, Leclair-Visonneau L, Coron E, Bourreille A, Le Dily S, Vavasseur F, et al. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. *Acta Neuropathol Commun.* (2015) 3:12. doi: 10.1186/s40478-015-0196-0
182. Schwiertz A, Spiegel J, Dillmann U, Grundmann D, Bürmann J, Faßbender K, et al. Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease. *Parkinsonism Relat Disord.* (2018) 50:104–7. doi: 10.1016/j.parkreldis.2018.02.022
183. Weimers P, Halfvarson J, Sachs MC, Saunders-Pullman R, Ludvigsson JF, Peter I, et al. Inflammatory bowel disease and Parkinson's disease: a nationwide Swedish Cohort Study. *Inflamm Bowel Dis.* (2018) 25:111–23. doi: 10.1093/ibd/izy190
184. Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T. Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977-2014. *Gut.* (2018) 68:18–24. doi: 10.1136/gutjnl-2017-315666
185. Peter I, Dubinsky M, Bressman S, Park A, Lu C, Chen N, et al. Anti-tumor necrosis factor therapy and incidence of Parkinson disease among patients with inflammatory bowel disease. *JAMA Neurol.* (2018) 75:939–46. doi: 10.1001/jamaneurol.2018.0605
186. Lin JC, Lin CS, Hsu CW, Lin CL, Kao CH. Association between Parkinson's disease and inflammatory bowel disease: a nationwide Taiwanese retrospective cohort study. *Inflamm Bowel Dis.* (2016) 22:1049–55. doi: 10.1097/MIB.0000000000000735
187. Zhu F, Li C, Gong J, Zhu W, Gu L, Li N. The risk of Parkinson's disease in inflammatory bowel disease: a systematic review and meta-analysis. *Dig Liver Dis.* (2019) 51:38–42. doi: 10.1016/j.dld.2018.09.017
188. Villarán RF, Espinosa-Oliva AM, Sarmiento M, De Pablos RM, Argüelles S, Delgado-Cortés MJ, et al. Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson's disease. *J Neurochem.* (2010) 114:1687–700. doi: 10.1111/j.1471-4159.2010.06879.x
189. Kishimoto Y, Zhu W, Hosoda W, Sen JM, Mattson MP. Chronic mild gut inflammation accelerates brain neuropathology and motor dysfunction in alpha-synuclein mutant mice. *Neuromolecular Med.* (2019) 21:239–49. doi: 10.1007/s12017-019-08539-5

190. Camacho-Soto A, Gross A, Searles Nielsen S, Dey N, Racette BA. Inflammatory bowel disease and risk of Parkinson's disease in Medicare beneficiaries. *Parkinsonism Relat Disord.* (2018) 50:23–28. doi: 10.1016/j.parkreldis.2018.02.008
191. Xiong Y, Dawson TM, Dawson VL. Models of LRRK2-associated Parkinson's disease. *Adv Neurobiol.* (2017) 14:163–91. doi: 10.1007/978-3-319-49969-7_9
192. Kowalski K, Mulak A. Brain-gut-microbiota axis in Alzheimer's disease. *J Neurogastroenterol Motil.* (2019) 25:48–60. doi: 10.5056/jnm18087
193. Zhang R, Miller RG, Gascon R, Champion S, Katz J, Lancero M, et al. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). *J Neuroimmunol.* (2009) 206:121–4. doi: 10.1016/j.jneuroim.2008.09.017
194. Zhao Y, Cong L, Jaber V, Lukiw WJ. Microbiome-derived lipopolysaccharide enriched in the perinuclear region of Alzheimer's disease brain. *Front Immunol.* (2017) 8:1064. doi: 10.3389/fimmu.2017.01064
195. Rera M, Clark RI, Walker DW. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. *Proc Natl Acad Sci USA.* (2012) 109:21528–33. doi: 10.1073/pnas.1215849110
196. Lustgarten MS. Classifying aging as a disease: the role of microbes. *Front Genet.* (2016) 7:212. doi: 10.3389/fgene.2016.00212
197. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia.* (2007) 55:453–62. doi: 10.1002/glia.20467
198. de Kivit S, Tobin MC, Forsyth CB, Keshavarzian A, Landay AL. Regulation of intestinal immune responses through TLR activation: implications for pro- and prebiotics. *Front Immunol.* (2014) 5:60. doi: 10.3389/fimmu.2014.00060
199. Park BS, Lee JO. Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp Mol Med.* (2013) 45:e66. doi: 10.1038/emm.2013.97
200. Liu Y, Qin L, Wilson B, Wu X, Qian L, Granholm AC, et al. Endotoxin induces a delayed loss of TH-IR neurons in substantia nigra and motor behavioral deficits. *Neurotoxicology.* (2008) 29:864–70. doi: 10.1016/j.neuro.2008.02.014
201. Caputi V, Giron MC. Microbiome-gut-brain axis and toll-like receptors in Parkinson's disease. *Int J Mol Sci.* (2018) 19:E1689. doi: 10.3390/ijms19061689
202. Noelker C, Morel L, Lescot T, Osterloh A, Alvarez-Fischer D, Breloer M, et al. Toll like receptor 4 mediates cell death in a mouse MPTP model of Parkinson disease. *Sci Rep.* (2013) 3:1393. doi: 10.1038/srep01393
203. Köhler CA, Maes M, Slyepchenko A, Berk M, Solmi M, Lanctôt KL, et al. The gut-brain axis, including the microbiome, leaky gut and bacterial translocation: mechanisms and pathophysiological role in Alzheimer's disease. *Curr Pharm Des.* (2016) 22:6152–66. doi: 10.2174/1381612822666160907093807
204. Buford TW, Carter CS, VanDerPol WJ, Chen D, Lefkowitz EJ, Eipers P, et al. Composition and richness of the serum microbiome differ by age and link to systemic inflammation. *Geroscience.* (2018) 40:257–68. doi: 10.1007/s11357-018-0026-y
205. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes.* (2008) 57:1470–81. doi: 10.2337/db07-1403
206. Song L, Pei L, Yao S, Wu Y, Shang Y. NLRP3 inflammasome in neurological diseases, from functions to therapies. *Front Cell Neurosci.* (2017) 11:63. doi: 10.3389/fncel.2017.00063
207. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, therapeutics. *Nat Med.* (2015) 21:677–87. doi: 10.1038/nm.3893
208. Freeman LC, Ting JP. The pathogenic role of the inflammasome in neurodegenerative diseases. *J Neurochem.* (2015) 136(Suppl. 1):29–38. doi: 10.1111/jnc.13217
209. He Y, Hara H, Nunez G. Mechanism and regulation of NLRP3 inflammasome activation. *Trends Biochem Sci.* (2016) 41:1012–1021. doi: 10.1016/j.tibs.2016.09.002
210. Walsh JG, Muruve DA, Power C. Inflammasomes in the CNS. *Nat Rev Neurosci.* (2014) 15:84–97. doi: 10.1038/nrn3638
211. Schroder K, Zhou R, Tschopp J. The NLRP3 inflammasome: a sensor for metabolic danger? *Science.* (2010) 327:296–300. doi: 10.1126/science.1184003
212. Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, et al. Triggering of inflammasome by aggregated alpha-synuclein, an inflammatory response in synucleinopathies. *PLoS ONE.* (2013) 8:e55375. doi: 10.1371/journal.pone.0055375
213. Elliott EI, Sutterwala FS. Initiation and perpetuation of NLRP3 inflammasome activation and assembly. *Immunol Rev.* (2015) 265:35–52. doi: 10.1111/imr.12286
214. Lee E, Hwang I, Park S, Hong S, Hwang B, Cho Y, et al. MPTP-driven NLRP3 inflammasome activation in microglia plays a central role in dopaminergic neurodegeneration. *Cell Death Differ.* (2018) 26:213–28. doi: 10.1038/s41418-018-0124-5
215. Sarkar S, Malovic E, Harishchandra DS, Ghaisas S, Panicker N, Charli A, et al. Mitochondrial impairment in microglia amplifies NLRP3 inflammasome proinflammatory signaling in cell culture and animal models of Parkinson's disease. *NPJ Parkinsons Dis.* (2017) 3:30. doi: 10.1038/s41531-017-0032-2
216. Giacoppo S, Bramanti P, Mazzon E. Triggering of inflammasome by impaired autophagy in response to acute experimental Parkinson's disease: involvement of the PI3K/Akt/mTOR pathway. *Neuroreport.* (2017) 28:996–1007. doi: 10.1097/WNR.0000000000000871
217. Gong Z, Pan J, Shen Q, Li M, Peng Y. Mitochondrial dysfunction induces NLRP3 inflammasome activation during cerebral ischemia/reperfusion injury. *J Neuroinflammation.* (2018) 15:242. doi: 10.1186/s12974-018-1282-6
218. Youm YH, Grant RW, McCabe LR, Albarado DC, Nguyen KY, Ravussin A, et al. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab.* (2013) 18:519–32. doi: 10.1016/j.cmet.2013.09.010
219. Heneka MT. Inflammasome activation and innate immunity in Alzheimer's disease. *Brain Pathol.* (2017) 27:220–2. doi: 10.1111/bpa.12483
220. Anderson FL, Coffey MM, Berwin BL, Havrda MC. Inflammasomes: an emerging mechanism translating environmental toxicant exposure into neuroinflammation in Parkinson's disease. *Toxicol Sci.* (2018) 166:3–15. doi: 10.1093/toxsci/kfy219
221. Chakraborty S, Kaushik DK, Gupta M, Basu A. Inflammasome signaling at the heart of central nervous system pathology. *J Neurosci Res.* (2010) 88:1615–31. doi: 10.1002/jnr.22343
222. Pavillard LE, Cañadas-Lozano D, Alcocer-Gómez E, Marín-Aguilar F, Pereira S, Robertson AAB, et al. NLRP3-inflammasome inhibition prevents high fat and high sugar diets-induced heart damage through autophagy induction. *Oncotarget.* (2017) 8:99740–56. doi: 10.18632/oncotarget.20763
223. Jena PK, Sheng L, Di Lucente J, Jin LW, Maezawa I, Wan YY. Dysregulated bile acid synthesis and dysbiosis are implicated in Western diet-induced systemic inflammation, microglial activation, reduced neuroplasticity. *FASEB J.* (2018) 32:2866–77. doi: 10.1096/fj.201700984RR
224. Guo C, Xie S, Chi Z, Zhang J, Liu Y, Zhang L, et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. *Immunity.* (2016) 45:802–16. doi: 10.1016/j.immuni.2016.10.009
225. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med.* (2011) 17:179–88. doi: 10.1038/nm.2279
226. Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, et al. Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc Natl Acad Sci USA.* (2011) 108:15324–9. doi: 10.1073/pnas.1100255108
227. Bloom GS, Lazo JS, Norambuena A. Reduced brain insulin signaling: a seminal process in Alzheimer's disease pathogenesis. *Neuropharmacology.* (2017) 136(Pt B):192–5. doi: 10.1016/j.neuropharm.2017.09.016
228. Athauda D, Foltynie T. Insulin resistance and Parkinson's disease: a new target for disease modification? *Prog Neurobiol.* (2016) 145–146:98–120. doi: 10.1016/j.pneurobio.2016.10.001
229. Aviles-Olmos I, Limousin P, Lees A, Foltynie T. Parkinson's disease, insulin resistance and novel agents of neuroprotection. *Brain.* (2013) 136(Pt 2):374–84. doi: 10.1093/brain/aws009
230. Wang J, Jia H. Metagenome-wide association studies: fine-mining the microbiome. *Nat Rev Microbiol.* (2016) 14:508–22. doi: 10.1038/nrmicro.2016.83
231. De Pablo-Fernandez E, Goldacre R, Pakpoor J, Noyce AJ, Warner TT. Association between diabetes and subsequent Parkinson disease:

a record-linkage cohort study. *Neurology*. (2018) 91:e139–42. doi: 10.1212/WNL.0000000000005771
232. Alters SE, McLaughlin B, Spink B, Lachinyan T, Wang CW, Podust V, et al. Integrative network analysis unveils convergent molecular pathways in Parkinson's disease and diabetes. *PLoS ONE*. (2013) 8:e83940. doi: 10.1371/journal.pone.0083940
233. Wahlqvist ML, Lee MS, Hsu CC, Chuang SY, Lee JT, Tsai HN. Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with Type 2 diabetes in a Taiwanese population cohort. *Parkinsonism Relat Disord*. (2012) 18:753–8. doi: 10.1016/j.parkreldis.2012.03.010
234. Hu G, Jousilahti P, Bidel S, Antikainen R, Tuomilehto J. Type 2 diabetes and the risk of Parkinson's disease. *Diabetes Care*. (2007) 30:842–7. doi: 10.2337/dc06-2011
235. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*. (2013) 498:99–103. doi: 10.1038/nature12198
236. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. (2012) 490:55–60. doi: 10.1038/nature11450
237. Coll RC, Robertson AA, Chae JJ, Higgins SC, Muñoz-Planillo R, Inserra MC, et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat Med*. (2015) 21:248–55. doi: 10.1038/nm.3806
238. Chen L, Xue L, Zheng J, Tian X, Zhang Y, Tong Q. PPARss/delta agonist alleviates NLRP3 inflammasome-mediated neuroinflammation in the MPTP mouse model of Parkinson's disease. *Behav Brain Res*. (2018) 356:483–9. doi: 10.1016/j.bbr.2018.06.005
239. Bian F, Xiao Y, Zaheer M, Volpe EA, Pflugfelder SC, Li DQ, et al. Inhibition of NLRP3 inflammasome pathway by butyrate improves corneal wound healing in corneal alkali burn. *Int J Mol Sci*. (2017) 18:E562. doi: 10.3390/ijms18030562
240. Abderrazak A, El Hadri K, Bosc E, Blondeau B, Slimane MN, Büchele B, et al. Inhibition of the inflammasome NLRP3 by arglabin attenuates inflammation, protects pancreatic beta-cells from apoptosis, prevents type 2 diabetes mellitus development in ApoE2Ki mice on a chronic high-fat diet. *J Pharmacol Exp Ther*. (2016) 357:487–94. doi: 10.1124/jpet.116.232934
241. Bodnaruc AM, Prud'homme D, Blanchet R, Giroux I. Nutritional modulation of endogenous glucagon-like peptide-1 secretion: a review. *Nutr Metab*. (2016) 13:92. doi: 10.1186/s12986-016-0153-3
242. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes*. (2011) 61:364–71. doi: 10.2337/db11-1019
243. Tolhurst G, Reimann F, Gribble FM. Nutritional regulation of glucagon-like peptide-1 secretion. *J Physiol*. (2009) 587:27–32. doi: 10.1113/jphysiol.2008.164012
244. Chen A, Chen Z, Xia Y, Lu D, Yang X, Sun A, et al. Liraglutide attenuates NLRP3 inflammasome-dependent pyroptosis via regulating SIRT1/NOX4/ROS pathway in H9c2 cells. *Biochem Biophys Res Commun*. (2018) 499:267–72. doi: 10.1016/j.bbrc.2018.03.142
245. Dai Y, Dai D, Wang X, Ding Z, Mehta JL. DPP-4 inhibitors repress NLRP3 inflammasome and interleukin-1beta via GLP-1 receptor in macrophages through protein kinase C pathway. *Cardiovasc Drugs Ther*. (2014) 28:425–32. doi: 10.1007/s10557-014-6539-4
246. Kim S, Moon M, Park S, Exendin-4 protects dopaminergic neurons by inhibition of microglial activation and matrix metalloproteinase-3 expression in an animal model of Parkinson's disease. *J Endocrinol*. (2009) 202:431–9. doi: 10.1677/JOE-09-0132
247. Holscher C. Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. *J Endocrinol*. (2014) 221:T31–41. doi: 10.1530/JOE-13-0221
248. Kim DS, Choi HI, Wang Y, Luo Y, Hoffer BJ, Greig NH. A new treatment strategy for Parkinson's disease through the gut-brain axis: the glucagon-like peptide-1 receptor pathway. *Cell Transplant*. (2017) 26:1560–71. doi: 10.1177/0963689717721234
249. Katsurada K, Yada T. Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist. *J Diabetes Investig*. (2016) 7(Suppl. 1):64–9. doi: 10.1111/jdi.12464
250. Mercado NM, Collier TJ, Sortwell CE, Steece-Collier K. BDNF in the aged brain: translational implications for Parkinson's disease. *Austin Neurol Neurosci*. (2017) 2:1021.
251. Wang Y, Liu H, Zhang BS, Soares JC, Zhang XY. Low BDNF is associated with cognitive impairments in patients with Parkinson's disease. *Parkinsonism Relat Disord*. (2016) 29:66–71. doi: 10.1016/j.parkreldis.2016.05.023
252. Yamane S, Inagaki N. Regulation of glucagon-like peptide-1 sensitivity by gut microbiota dysbiosis. *J Diabetes Investig*. (2017) 9:262–4. doi: 10.1111/jdi.12762
253. Kappe C, Tracy LM, Patrone C, Iverfeldt K, Sjöholm Å. GLP-1 secretion by microglial cells and decreased CNS expression in obesity. *J Neuroinflammation*. (2012) 9:276. doi: 10.1186/1742-2094-9-276
254. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*. (2011) 141:599–609, 609.e1–3. doi: 10.1053/j.gastro.2011.04.052
255. Feng P, Zhang X, Li D, Ji C, Yuan Z, Wang R, et al. Two novel dual GLP-1/GIP receptor agonists are neuroprotective in the MPTP mouse model of Parkinson's disease. *Neuropharmacology*. (2018) 133:385–94. doi: 10.1016/j.neuropharm.2018.02.012
256. Ji C, Xue G-F, Lijun C, Feng P, Li D, Li G, et al. A novel dual GLP-1 and GIP receptor agonist is neuroprotective in the MPTP mouse model of Parkinson's disease by increasing expression of BND. *Brain Res*. (2015) 1634:1–11. doi: 10.1016/j.brainres.2015.09.035
257. Athauda D, Foltynie T. Protective effects of the GLP-1 mimetic exendin-4 in Parkinson's disease. *Neuropharmacology*. (2018) 136(Pt B):260–70. doi: 10.1016/j.neuropharm.2017.09.023
258. Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. *Lancet*. (2017) 390:1664–75. doi: 10.1016/S0140-6736(17)31585-4
259. Cani PD, Everard A. Talking microbes: When gut bacteria interact with diet and host organs. *Mol Nutr Food Res*. (2016) 60:58–66. doi: 10.1002/mnfr.201500406
260. O'Leary OF, Ogbonnaya ES, Felice D, Levone BR, C Conroy L, Fitzgerald P, et al. The vagus nerve modulates BDNF expression and neurogenesis in the hippocampus. *Eur Neuropsychopharmacol*. (2018) 28:307–316. doi: 10.1016/j.euroneuro.2017.12.004
261. Buchman AS, Yu L, Boyle PA, Schneider JA, De Jager PL, Bennett DA. Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. *Neurology*. (2016) 86:735–41. doi: 10.1212/WNL.0000000000002387
262. Motamedi S, Karimi I, Jafari F. The interrelationship of metabolic syndrome and neurodegenerative diseases with focus on brain-derived neurotrophic factor (BDNF): kill two birds with one stone. *Metab Brain Dis*. (2014) 32:651–65. doi: 10.1007/s11011-017-9997-0
263. Barichello T, Generoso JS, Simões LR, Faller CJ, Ceretta RA, Petronilho F, et al. Sodium butyrate prevents memory impairment by re-establishing BDNF and GDNF expression in experimental pneumococcal meningitis. *Mol Neurobiol*. (2015) 52:734–40. doi: 10.1007/s12035-014-8914-3
264. Howells DW, Porritt MJ, Wong JY, Batchelor PE, Kalnins R, Hughes AJ, et al. Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp Neurol*. (2000) 166:127–35. doi: 10.1006/exnr.2000.7483
265. Rahmani F, Saghazadeh A, Rahmani M, Teixeira AL, Rezaei N, Aghamollaii V, et al. Plasma levels of brain-derived neurotrophic factor in patients with Parkinson disease: a systematic review and meta-analysis. *Brain Res*. (2018) 1704:127–36. doi: 10.1016/j.brainres.2018.10.006
266. Maqsood R, Stone TW. The gut-brain axis, BDNF, NMDA and CNS disorders. *Neurochem Res*. (2016) 41:2819–35. doi: 10.1007/s11064-016-2039-1
267. Sen T, Cawthon CR, Ihde BT, Hajnal A, DiLorenzo PM, de La Serre CB, et al. Diet-driven microbiota dysbiosis is associated with vagal remodeling and obesity. *Physiol Behav*. (2017) 173:305–17. doi: 10.1016/j.physbeh.2017.02.027

268. Erro R, Brigo F, Tamburin S, Zamboni M, Antonini A, Tinazzi M. Nutritional habits, risk, progression of Parkinson disease. *J Neurol.* (2017) 265:12–23. doi: 10.1007/s00415-017-8639-0
269. Gao X, Chen H, Fung TT, Logroscino G, Schwarzschild MA, Hu FB, et al. Prospective study of dietary pattern and risk of Parkinson disease. *Am J Clin Nutr.* (2007) 86:1486–94. doi: 10.1093/ajcn/86.5.1486
270. Alcalay RN, Gu Y, Mejia-Santana H, Cote L, Marder KS, Scarmeas N. The association between Mediterranean diet adherence and Parkinson's disease. *Mov Disord.* (2012) 27:771–4. doi: 10.1002/mds.24918
271. David LA, Materna AC, Friedman J, Campos-Baptista MI, Blackburn MC, Perrotta A, et al. Host lifestyle affects human microbiota on daily timescales. *Genome Biol.* (2014) 15:R89. doi: 10.1186/gb-2014-15-7-r89
272. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol.* (2015) 11:577–91. doi: 10.1038/nrendo.2015.128
273. Canfora EE, van der Beek CM, Jocken JWE, Goossens GH, Holst JJ, Olde Damink SWM, et al. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci Rep.* (2017) 7:2360. doi: 10.1038/s41598-017-02546-x
274. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab.* (2009) 9:311–26. doi: 10.1016/j.cmet.2009.02.002
275. Tozser J, Benko S. Natural compounds as regulators of NLRP3 inflammasome-mediated IL-1β production. *Mediators Inflamm.* (2016) 2016:5460302. doi: 10.1155/2016/5460302
276. Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. *Ageing Res Rev.* (2016) 39:46–58. doi: 10.1016/j.arr.2016.10.005
277. Mattson MP, Arumugam TV. Hallmarks of brain aging: adaptive and pathological modification by metabolic states. *Cell Metab.* (2018) 27:1176–99. doi: 10.1016/j.cmet.2018.05.011
278. Mattson MP, Moehl K, Ghena N, Schmaedick M, Cheng A. Intermittent metabolic switching, neuroplasticity and brain health. *Nat Rev Neurosci.* (2018) 19:63–80. doi: 10.1038/nrn.2017.156
279. Norwitz NG, Hu MT, Clarke K. The mechanisms by which the ketone body D-β-hydroxybutyrate may improve the multiple cellular pathologies of Parkinson's disease. *Front Nutr.* (2019) 6:63. doi: 10.3389/fnut.2019.00063
280. Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. *Cell Metab.* (2014) 19:181–92. doi: 10.1016/j.cmet.2013.12.008
281. Duan W, Mattson MP. Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J Neurosci Res.* (1999) 57:195–206.
282. Maswood N, Young J, Tilmont E, Zhang Z, Gash DM, Gerhardt GA, et al. Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. *Proc Natl Acad Sci USA.* (2004) 101:18171–6. doi: 10.1073/pnas.0405831102
283. Maalouf M, Rho JM, Mattson MP. The neuroprotective properties of calorie restriction, the ketogenic diet, ketone bodies. *Brain Res Rev.* (2009) 59:293–315. doi: 10.1016/j.brainresrev.2008.09.002
284. Vanitallie TB, Nonas C, Di Rocco A, Boyar K, Hyams K, Heymsfield SB. Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study. *Neurology.* (2005) 64:728–30. doi: 10.1212/01.WNL.0000152046.11390.45
285. Cornille E, Abou-Hamdan M, Khrestchatisky M, Nieoullon A, de Reggi M, Gharib B. Enhancement of L-3-hydroxybutyryl-CoA dehydrogenase activity and circulating ketone body levels by pantethine. Relevance to dopaminergic injury. *BMC Neurosci.* (2010) 11:51. doi: 10.1186/1471-2202-11-51
286. Tieu K, Perier C, Caspersen C, Teismann P, Wu DC, Yan SD, et al. D-β-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *J Clin Invest.* (2003) 112:892–901. doi: 10.1172/JCI200318797
287. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, et al. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med.* (2015) 21:263–9. doi: 10.1038/nm.3804
288. Traba J, Geiger SS, Kwarteng-Siaw M, Han K, Ra OH, Siegel RM, et al. Prolonged fasting suppresses mitochondrial NLRP3 inflammasome assembly and activation via SIRT3-mediated activation of superoxide dismutase 2. *J Biol Chem.* (2017) 292:12153–64. doi: 10.1074/jbc.M117.791715
289. Traba J, Kwarteng-Siaw M, Okoli TC, Li J, Huffstutler RD, Bray A, et al. Fasting and refeeding differentially regulate NLRP3 inflammasome activation in human subjects. *J Clin Invest.* (2015) 125:4592–600. doi: 10.1172/JCI83260
290. Goldberg EL, Asher JL, Molony RD, Shaw AC, Zeiss CJ, Wang C, et al. β-hydroxybutyrate deactivates neutrophil NLRP3 inflammasome to relieve gout flares. *Cell Rep.* (2017) 18:2077–87. doi: 10.1016/j.celrep.2017.02.004
291. Li G, Xie C, Lu S, Nichols RG, Tian Y, Li L, et al. Intermittent fasting promotes white adipose browning and decreases obesity by shaping the gut microbiota. *Cell Metab.* (2017) 26:672–685.e4. doi: 10.1016/j.cmet.2017.08.019
292. Beli E, Yan Y, Moldovan L, Vieira CP, Gao R, Duan Y, et al. Restructuring of the gut microbiome by intermittent fasting prevents retinopathy and prolongs survival in db/db mice. *Diabetes.* (2018) 67:1867–79. doi: 10.2337/db18-0158
293. Moon M, Kim HG, Hwang L, Seo JH, Kim S, Hwang S, et al. Neuroprotective effect of ghrelin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease by blocking microglial activation. *Neurotox Res.* (2009) 15:332–47. doi: 10.1007/s12640-009-9037-x
294. Bayliss JA, Andrews ZB. Ghrelin is neuroprotective in Parkinson's disease: molecular mechanisms of metabolic neuroprotection. *Ther Adv Endocrinol Metab.* (2013) 4:25–36. doi: 10.1177/2042018813479645
295. Andrews ZB, Erion D, Beiler R, Liu ZW, Abizaid A, Zigman J, et al. Ghrelin promotes and protects nigrostriatal dopamine function via a UCP2-dependent mitochondrial mechanism. *J Neurosci.* (2009) 29:14057–65. doi: 10.1523/JNEUROSCI.3890-09.2009
296. Dyall SC. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. *Front Aging Neurosci.* (2015) 7:52. doi: 10.3389/fnagi.2015.00052
297. da Silva TM, Munhoz RP, Alvarez C, Naliwaiko K, Kiss A, Andreatini R, et al. Depression in Parkinson's disease: a double-blind, randomized, placebo-controlled pilot study of omega-3 fatty-acid supplementation. *J Affect Disord.* (2008) 111:351–9. doi: 10.1016/j.jad.2008.03.008
298. Luchtman DW, Meng Q, Song C. Ethyl-eicosapentaenoate (E-EPA) attenuates motor impairments and inflammation in the MPTP-probenecid mouse model of Parkinson's disease. *Behav Brain Res.* (2012) 226:386–96. doi: 10.1016/j.bbr.2011.09.033
299. Taepavarapruk P, Song C. Reductions of acetylcholine release and nerve growth factor expression are correlated with memory impairment induced by interleukin-1β administrations: effects of omega-3 fatty acid EPA treatment. *J Neurochem.* (2010) 112:1054–64. doi: 10.1111/j.1471-4159.2009.06524.x
300. Meng Q, Luchtman DW, El Bahh B, Zidichouski JA, Yang J, Song C. Ethyl-eicosapentaenoate modulates changes in neurochemistry and brain lipids induced by parkinsonian neurotoxin 1-methyl-4-phenylpyridinium in mouse brain slices. *Eur J Pharmacol.* (2010) 649:127–34. doi: 10.1016/j.ejphar.2010.09.046
301. Luchtman DW, Meng Q, Wang X, Shao D, Song C. omega-3 fatty acid eicosapentaenoic acid attenuates MPP+-induced neurodegeneration in fully differentiated human SH-SY5Y and primary mesencephalic cells. *J Neurochem.* (2013) 124:855–68. doi: 10.1111/jnc.12068
302. Cansev M, Ulus IH, Wang L, Maher TJ, Wurtman RJ. Restorative effects of uridine plus docosahexaenoic acid in a rat model of Parkinson's disease. *Neurosci Res.* (2008) 62:206–9. doi: 10.1016/j.neures.2008.07.005
303. Perez-Pardo P, de Jong EM, Broersen LM, van Wijk N, Attali A, Garssen J, et al. Promising effects of neurorestorative diets on motor, cognitive, gastrointestinal dysfunction after symptom development in a mouse model of Parkinson's disease. *Front Aging Neurosci.* (2017) 9:57. doi: 10.3389/fnagi.2017.00057
304. Ozsoy O, Seval-Celik Y, Hacioglu G, Yargicoglu P, Demir R, Agar A, et al. The influence and the mechanism of docosahexaenoic acid on a mouse model of Parkinson's disease. *Neurochem Int.* (2011) 59:664–70. doi: 10.1016/j.neuint.2011.06.012
305. Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem N, et al. Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA

receptors in the brain of a transgenic mouse model of Alzheimer's disease. *Eur J Neurosci.* (2005) 22:617–26. doi: 10.1111/j.1460-9568.2005.04253.x
306. Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L, et al. Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-beta and tau pathology via a mechanism involving presenilin 1 levels. *J Neurosci.* (2007) 27:4385–95. doi: 10.1523/JNEUROSCI.0055-07.2007
307. Sakamoto T, Cansev M, Wurtman RJ. Oral supplementation with docosahexaenoic acid and uridine-5'-monophosphate increases dendritic spine density in adult gerbil hippocampus. *Brain Res.* (2007) 1182:50–9. doi: 10.1016/j.brainres.2007.08.089
308. Serhan CN, Petasis NA. Resolvins and protectins in inflammation resolution. *Chem Rev.* (2011) 111:5922–43. doi: 10.1021/cr100396c
309. Bazan NG. Cellular and molecular events mediated by docosahexaenoic acid-derived neuroprotectin D1 signaling in photoreceptor cell survival and brain protection. *Prostaglandins Leukot Essent Fatty Acids.* (2009) 81:205–11. doi: 10.1016/j.plefa.2009.05.024
310. Ren J, Meng S, Yan B, Yu J, Liu J. Protectin D1 reduces concanavalin A-induced liver injury by inhibiting NF-kappaB-mediated CX3CL1/CX3CR1 axis and NLR family, pyrin domain containing 3 inflammasome activation. *Mol Med Rep.* (2016) 13:3627–38. doi: 10.3892/mmr.2016.4980
311. Lin C, Chao H, Li Z, Xu X, Liu Y, Bao Z, et al. Omega-3 fatty acids regulate NLRP3 inflammasome activation and prevent behavior deficits after traumatic brain injury. *Exp Neurol.* (2017) 290:115–122. doi: 10.1016/j.expneurol.2017.01.005
312. Harvey LD, Yin Y, Attarwala IY, Begum G, Deng J, Yan HQ, et al. Administration of DHA reduces endoplasmic reticulum stress-associated inflammation and alters microglial or macrophage activation in traumatic brain injury. *ASN Neuro.* (2015) 7:1759091415618969. doi: 10.1177/1759091415618969
313. Sui YH, Luo WJ, Xu QY, Hua J. Dietary saturated fatty acid and polyunsaturated fatty acid oppositely affect hepatic NOD-like receptor protein 3 inflammasome through regulating nuclear factor-kappa B activation. *World J Gastroenterol.* (2016) 22:2533–44. doi: 10.3748/wjg.v22.i8.2533
314. Yan Y, Jiang W, Spinetti T, Tardivel A, Castillo R, Bourquin C, et al. Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. *Immunity.* (2013) 38:1154–63. doi: 10.1016/j.immuni.2013.05.015
315. Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, et al. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron.* (2004) 43:633–45. doi: 10.1016/j.neuron.2004.08.013
316. Wu A, Ying Z, Gomez-Pinilla F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, counteract learning disability after traumatic brain injury in rats. *J Neurotrauma.* (2004) 21:1457–67. doi: 10.1089/neu.2004.21.1457
317. Hashimoto M, Tanabe Y, Fujii Y, Kikuta T, Shibata H, Shido O. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid beta-infused rats. *J Nutr.* (2005) 135:549–55. doi: 10.1093/jn/135.3.549
318. Moriguchi T, Greiner RS, Salem N Jr. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. *J Neurochem.* (2000) 75:2563–73. doi: 10.1046/j.1471-4159.2000.0752563.x
319. de Urquiza AM, Liu S, Sjöberg M, Zetterström RH, Griffiths W, Sjövall J, et al. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science.* (2000) 290:2140–4. doi: 10.1126/science.290.5499.2140

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Gut–Brain Axis: Potential Factors Involved in the Pathogenesis of Parkinson's Disease

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Increasing evidence suggests an association between gastrointestinal (GI) disorders and susceptibility and progress of Parkinson's disease (PD). Gut–brain axis has been proposed to play important roles in the pathogenesis of PD, though the exact pathophysiologic mechanism has yet to be elucidated. Here, we discuss the common factors involved in both PD and GI disorders, including genes, altered gut microbiota, diet, environmental toxins, and altered mucosal immunity. Large-scale prospective clinical studies are needed to define the exact relationship between dietary factors, microbiome, and genetic factors in PD. Identification of early diagnostic markers and demonstration of the efficacy of diet modulation and regulation of gut microbiome through specific therapeutics can potentially change the treatment paradigm for PD.

Keywords: Parkinson's disease (PD), gut, genetics, microbiome, diet

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INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder affecting 1–2 per 1,000 of the population (1). The incidence rate is generally lower for individuals before the age of 50 years, and it increases steadily with advanced age, peaking at 80 years old (2). The pathological hallmark in PD is the presence of intraneuronal aggregated alpha-synuclein (α-syn), Lewy body formation, and progressive loss of dopaminergic neurons in the substantia nigra compacta (SNc) which leads to the typical clinical symptoms including tremor, rigidity, bradykinesia, and posture instability (1). Current treatment for PD is largely symptomatic.

Although motor symptoms are characteristic in PD, non-motor abnormalities in pre-PD phase are increasingly recognized. Among those, constipation is a prodromal marker in research diagnostic criteria for PD and may be an early manifestation of PD pathophysiology (3–5). The extent of the observed severity of the manifestation, especially the duration preceding PD, is unclear (3). However, several studies associate gastrointestinal (GI) dysfunction as a risk factor for PD development, with an early prevalence of 20% pre-PD diagnosis and 50% of the PD cases post-diagnosis (6, 7). Moreover, the association with GI dysfunction corroborates the well-established Braak's theory that PD initiation might begin in the GI tract, supported by the presence of Lewy body burden in the enteric nervous system (ENS) compared with other body regions and in the central nervous system (CNS) (8, 9). This has led to considerable interests to understand the etiology and presentation of pre-motor symptoms in PD patients. This review highlights the current findings linking pathophysiologic mechanisms between CNS and ENS in PD (**Figure 1**).

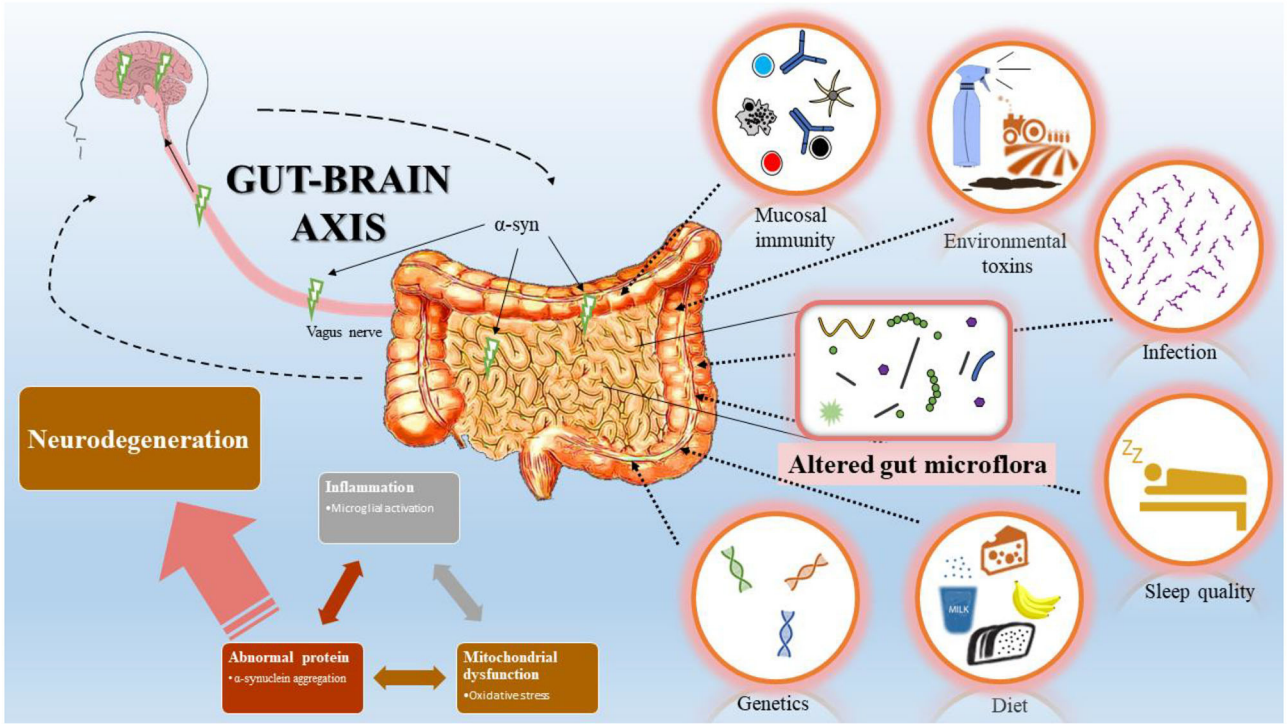

FIGURE 1 | Bi-directional interaction between gastrointestinal (GI) tract and central nervous system (CNS). Schematic representation summarizes Braak's model of Parkinson's disease (PD) progression initiated from the GI tract. Changes in GI mucosal immunity, environmental toxins, infection, sleep quality, diet, and genetics modify the gut microflora and induce inflammation, mitochondrial dysfunction, and abnormal protein accumulation. Accumulation of α-syn in the GI tract spread via the vagus nerve to the CNS and leads to dopaminergic neuron degeneration.

Braak's Hypothesis and α-Synuclein

Emerging evidences have shown that PD involves not only the brain but also outside the CNS including the GI system (10, 11). Some propose the idea of a prion-like spread whereas others believe that it involves an interplay of multiple complex molecular mechanisms, including the well-known Braak's dual-hit model (12–15). According to Braak et al., the bi-directional communication between the network of neurons in the GI tract and the neurons of the CNS forms the gut–brain axis (10). Though criticisms argue that not all PD patients have the specific α-syn spreading pattern proposed by Braak, Braak's hypothesis suggests disease initiation and progression in a systematic manner in sporadic PD (14).

Braak et al. initial suggestion was an involvement of a neurotrophic agent or an unknown pathogenic insult in the GI tract (9). They went on to propose a six-stage system of PD progression in the brain and surrounding olfactory regions based on observed α-syn spreading patterns (16), and this can be linked to the many clinical features, and motor and non-motor syndromes of Parkinsonism (17, 18). Moreover, evidence of α-syn aggregations at olfactory bulbs (OBs), the ENS, and submucosal plexuses was associated with different pathologies observed in PD (17). Further studies underline the fact that the invading neurotrophic agent may either be a GI-initiated trigger by the intestinal microbiota or a toxin/pathogen from an external environment entering through the olfactory route (9, 19). As a consequence, this invasion promotes a pro-inflammatory intestinal mucosal environment, increases intestinal barrier permeability, which leads to the accumulation of reactive oxygen species (ROS), and creates an unbalanced homeostasis activating various immune mechanisms, which may ultimately trigger α-syn aggregation (14). It was increasingly evident that the initiation and spreading projected from two pathways, olfactory and GI tract (20, 21). Projecting neurons create a path via the vagal nerve and the dorsal motor nucleus of the vagus nerve (DMV) in the medulla (21). The aggregated α-syn was postulated to ascend anterogradely from the OB and retrogradely from the plexus of the GI tract via the vagus nerve (21). The α-syn aggregates propagate trans-synaptically to the DMV and eventually other regions of the CNS (15, 16, 21).

Common Factors in the Pathogenesis of PD and Gastrointestinal Disorders

Here, we review potential factors involved in the association of GI disorders and PD, focusing on the common genetic factors, gut microbiota, and mucosal immunity. The environmental factors such as diet and environmental toxins together with potential role of sleep disorder will also be briefly discussed.

Genetic Factors

While most PD are sporadic with unknown etiologies, monogenic forms of PD and common genetic risk variants

in sporadic PD have been identified (1, 22, 23). Carriers of pathogenic gene mutations frequently have indistinguishable clinical presentation from non-carriers (24).

Leucine-rich repeat kinase 2 (LRRK-2) is the most common genetic cause of autosomal dominant PD, accounting for 10–40% of familial cases in different populations (25). Genome-wide association studies (GWAS) show that some PD-associated LRRK2 variants are also independently associated with inflammatory bowel diseases (IBDs) (1, 23, 26, 27). More than 100 putative mutations have been reported in LRRK2 gene, though only six have been consistently shown to cause diseases, with two of these mutations G2019S and R1441C most commonly reported (28). Among the many functions of LRRK-2, the key roles include α-syn clearance and regulating the inflammatory response (22).

The genetic basis for IBD, in particular, Crohn's disease (CD) and ulcerative colitis (UC), has been supported by GWAS, which also suggested that some GWAS loci may also be associated with risk for PD (27, 29–31). This may be caused by susceptible individuals having an impaired mucosal immune response to GI commensals (29, 32). A Danish study made a similar association between PD and IBD in their cohort comprising IBD and non-IBD population (22). Apart from immune involvement, the authors also observed prominent differences in the gut microbiota in both CD and UC patients (22). These changes may have enabled the formations of Lewy pathology observed in PD, which can eventually through gut–brain neuronal interactions spread throughout the body (14).

Gut Microbiota

The involvement of gut microbiota in α-syn aggregation in PD has received increasing attention in the past several years (33, 34). Sampson and colleagues had shown that orally giving microbial metabolites can cause neuroinflammation in germ-free mice which leads to motor symptom development (35). Remarkably, microbiota transplants from PD patients exaggerated motor symptoms in α-syn-overexpressing mice compared with healthy controls. Other studies also suggested the synergistic role of gut microbiota in α-syn pathophysiology and neurodegeneration (36).

Gram-negative bacterium Helicobacter pylori causes gastritis and various GI problems, especially peptic ulcers (37–39). The association between PD and *H. pylori* was highlighted by Altschuler who noted the presence of duodenal ulcers in many clinical situations and suggested a probable causal link with idiopathic PD (40). Meta-analyses comparing healthy and *H. pylori*–affected individuals demonstrate a clear association between *H. pylori* and PD (39, 41). However, disease progression can be multifactorial, and it is impossible to single out a direct cause. Several investigators proposed various mechanisms of action associating *H. pylori* with PD pathogenesis. First, it is possible that *H. pylori* could be releasing CNS toxins vacuolating toxin, Vag A, and cytotoxin-associated gene, Cag A (37). Second, the damage can be through *H. pylori*–mediated glycosylation to generate cholesteryl glucosides, similar in form to toxin cycads (37, 42). These cholesteryl glucosides are neurotoxic, and they cross the blood–brain barrier (BBB) to cause dopaminergic neuron degeneration (37). Third, *H. pylori* can activate immune mechanisms, monocytes, eicosanoids, interleukins, and cytokines (TNF-α, IL-10, IL-6, IL-8, IL-1B, IL-13), resulting in an exaggerated neuroinflammatory response, leading to disruption and infiltration in the BBB, microgliosis, and neurodegeneration (39). Fourth, *H. pylori* can initiate apoptosis through apoptotic pathways such as the nitric oxide and mitochondrial Fas–FasL pathway, causing neurodegeneration (39). Lastly, the production of autoantibodies against dopaminergic neurons induced by *H. pylori* and host antigens can lead to widespread neuroinflammation (37, 39).

More recently, Wallen et al. conducted an association study (MWAS) between microbiome and PD using two large datasets. They found that the opportunistic pathogens and carbohydrate-metabolizing probiotics were significantly increased while short-chain fatty acid (SCFA)–producing bacteria were decreased in PD patients (43). These findings will facilitate testing the potential role of some of these pathogens in PD pathogenesis.

Diet

The association between diet, nutritional status, and PD pathogenesis has also attracted considerable attention after studies on the existence of the gut–brain axis and gut microbiota (22). Reduction in gut commensal Prevotellaceae composition reduces mucin synthesis increasing gut leakiness, affecting the production of SCFA involved in thiamine and folate biosynthesis, and the increase in Lactobacilliceae can alter gut hormone ghrelin which can modify nigrostriatal dopamine neuronal integrity (19). SCFAs can also exert a systemic anti-inflammatory response increasing ROS, which can lead to synucleinopathy (14, 19).

Moreover, celiac disease, a gluten-induced gastrointestinal disorder, has been reported to be associated with PD pathogenesis. Based on the results from a pilot study, 2 out of 67 celiac disease patients from the cohort reported PD symptoms (44). When these patients underwent a diet alteration to a more gluten-free one, their symptoms improved (45). Although these studies are preliminary, further investigation should be conducted with a larger cohort to illustrate this association and the importance of diet in PD.

Mucosal Immunity

The intestinal lumen encompasses the most extensive enviro-host interface, continuously interrogated by a high antigenic load resulting from exposures to deadly pathogens, diet changes, and commensals (32). Existing immune systems and co-evolving microbial community are reciprocal, and there are mandatory checkpoints available to ensure an appropriate response to a pathogenic insult (46). These systems continue to regulate and shape its response, accommodating to the changes observed throughout the host's lifetime (46).

The cellular aspects of GALT and the epithelial barrier comprise the localized microenvironment, lymphoid follicles, mesenteric lymph nodes, and Peyer's and colonic patches, whereas the molecular compartment consists of T and B regulatory cells, intraepithelial lymphocytes (IELs), innate lymphoid cells, macrophages, and dendritic cells (46, 47). GALT,

especially the immune cells in the appendix, were recently found unique for PD pathogenesis (48). The epithelial barrier and the cells of the intestinal epithelium are the first lines of defense against any invading pathogen (32, 46). Its unique structure functions to provide a physical barrier, drawing a forefront rich with antimicrobial peptides, immunoglobulins A (IgA), and a tight monolayer preventing bacterial penetrations (32, 46). Although there were contradicting observations on the noticeable structural changes in a disease state, many agree that the most imminent damage occurs to the tight monolayer (49). Epithelial dysfunction demonstrated in 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) animal models demonstrated noticeable differences in expression patterns of ZO-1, occludin, and tight-junction proteins (32, 50). Indeed, colonic biopsies from PD individuals confirm this observation (50).

Regulatory cells (Tregs) are a subset of $CD4^+$ T cells that hamper the progression of IBDs and provide peripheral tolerance (32). Among the many functions of Tregs, one which is worth mentioning is its ability to act as a negative regulator, aimed at curtailing a pro-inflammatory situation presented by effector (Teff) cells (32). They achieve this by actively secreting cytokines (IL-10, TGF-β) and cytotoxic T-lymphocyte antigen, CTLA 4 (32).

It would be apt to describe the characteristic features of IBD as a disease with a defective T-cell signaling, mostly imbalances between Treg and Th17, along with an altered cytokine profile (32, 51). Both Th17 and Treg cells originate from a common $CD4^+$ precursor cell, mediated by TGF-β signal (52). However, their fates differ at the end stage of differentiation (52). As opposed to Treg's function of maintaining intestinal homeostasis, Th17 cells initiate gut inflammation (51). In addition, commensal microbiota and bacterial metabolites can also positively or negatively alter cytokine profiles, inducing the pathway toward Treg or Th1/Th17 formations (32, 52). Supporting this observation, independent findings on PD patients' colonic biopsies and inflammatory diseases both indicate an exaggerated inflammation with extreme amounts of pro-inflammatory (TNF, IL-1β, IFNγ, IL-5) molecules (22, 32). Co-culture of autologous Th17 cells and stem cell–derived dopaminergic (DA) neurons showed that Th17 cells can kill the DA neurons through releasing of IL-17A (35). Whether these DA neuron–specific Th17 cells are from the mucosal immunity is unknown.

There are other relevant cells of the immune system with a primary role to function constitutively with other immune cells to maintain homeostasis in PD. They provide a supportive role in ensuring inflammation control and immune surveillance. For instance, the intestinal epithelial cells (IECs) of the epithelium secrete IgA, antimicrobial proteins, and anti-inflammatory cytokines with crucial roles in differentiation, maturation, migration, and response (32). Similarly, another cell population found alongside IECs are the IELs (32, 47). IELs are T cells with a T-cell receptor which have come in contact with antigens and have differentiated in either natural IEL or induced IELs (32). Although they take on separate differentiation patterns, their central role is to maintain intestinal homeostasis (32). They secrete pro-inflammatory (IFNγ and TNF) cytokines, provide immune surveillance through migration to intestinal epithelial surface, which is in close contact with pathogens, and produce IL-10 and TGF-β suppressing intestinal inflammation (32). Likewise, regulatory B cells (Bregs), antibody-producing cells, which release cytokines (IL-10) are also involved in maintaining homeostasis and suppressing inflammation, and regulating the balance of Tregs, Th1, and Th17 (32).

The distinctive pattern of GI inflammation, especially at the early stages of the disease, with its signature symptoms, suggests the extent of the involvement of the mucosal immune system. It is unclear if α-syn aggregates were the cause or effect in the pathophysiology (53). Stolzenberg et al. concluded that α-syn secreted from enteric nerves of a pro-inflammatory ENS is the cause of GI inflammation, and it also acts as a chemoattractant for neutrophils and monocytes perpetuating the condition (53).

Environmental Toxins

The link between herbicide and paraquat exposure and neurotoxin MPTP administration and PD has suggested that environmental toxins can cause the disease. A recent meta-analysis from 31 studies with occupational exposure to pesticides suggested a significant association with PD risk (54). Rotenone has been reported to inhibit mitochondrial complex 1 activity, whereas paraquat causes oxidative stress (55–58). The gram-negative bacteria endotoxin lipopolysaccharides (LPS) have also been reported to induce dopaminergic neuron death in animal models (59–61). Supporting Braak's theory of a peripheral-to-central spread, agrochemicals such as metals, pesticides, and herbicides that enter the body via inhalation and/or ingestion are suggested to be a possible initiator causing widespread inflammation and mitochondrial dysfunction which ultimately lead to abnormal α-syn accumulation and dopaminergic neuron degeneration in the midbrain (10, 59, 62). Moreover, an established causal link between agrochemical use and PD can be challenging as the time between exposure and symptom presentation has a long latency period (10 to 20 years) (62). Hence, epidemiological studies have to improve their assessment methodologies, employ neurologists for diagnostics, and redefine the way they study past exposures accurately (63).

Sleep Quality

Sleep disorder is one of the non-motor symptoms reported in PD patients in the prodromal phase (64, 65). Interestingly, sleep disturbance has also been reported in IBD patients (66, 67). The underlying mechanisms for the sleep disturbance in PD and IBD are yet to be elucidated.

CONCLUSIONS AND FUTURE PERSPECTIVES

The etiology of PD involves both genetic and environmental factors. The gut is one of the major systems exposed to the environment directly and connects to the brain. Understanding the gut–brain axis has allowed us to appreciate the development and progression of the disease considerably. The GI system (which consists of the microbiome) is continuously being influenced by various factors, such as environment, diet, infection, and mucosal immunity. The overlapping genetic factors between PD and GI disorders suggest common etiologic

links between the GI system and PD development. Given that the current treatments for PD are mainly symptomatic, regulation of the gut microbiota and mucosal immunity through diet, such as giving probiotics, may have protective effect in PD treatment. The association of PD with GI system may provide prophylactic and targeted PD therapy in selected risk individuals.

Large-scale prospective clinical studies are needed to define the exact relationship between dietary factors, microbiome, and genetic factors in PD. Identification of early diagnostic markers and demonstration of the efficacy of diet modulation and regulation of gut microbiome through specific therapeutics can potentially change the treatment paradigm for PD.

AUTHOR CONTRIBUTIONS

Y-XC and E-KT planned the outline of the manuscript. MG prepared the draft. NC, Y-XC, E-KT, LF, and OR revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Tysnes OB, Storstein A. Epidemiology of Parkinson's disease. *J Neural Transm.* (2017) 124:901–5. doi: 10.1007/s00702-017-1686-y
2. Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* (2016) 15:1257–72. doi: 10.1016/S1474-4422(16)30230-7
3. Kujawska M, Jodynis-Liebert J. What is the evidence that Parkinson's disease is a prion disorder, which originates in the gut? *Int J Mol Sci.* (2018) 19:3573. doi: 10.3390/ijms19113573
4. Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, et al. MDS research criteria for prodromal Parkinson's disease. *Mov Disord.* (2015) 30:1600–11. doi: 10.1002/mds.26431
5. Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB, et al. Update of the MDS research criteria for prodromal Parkinson's disease. *Mov Disord.* (2019) 34:1464–70. doi: 10.1002/mds.27802
6. Adams-Carr KL, Bestwick JP, Shribman S, Lees A, Schrag A, Noyce AJ. Constipation preceding Parkinson's disease: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry.* (2016) 87:710–6. doi: 10.1136/jnnp-2015-311680
7. Knudsen K, Krogh K, Ostergaard K, Borghammer P. Constipation in Parkinson's disease: subjective symptoms, objective markers, and new perspectives. *Mov Disord.* (2017) 32:94–105. doi: 10.1002/mds.26866
8. Lin CH, Lin JW, Liu YC, Chang CH, Wu RM. Risk of Parkinson's disease following severe constipation: a nationwide population-based cohort study. *Parkinsonism Relat Disord.* (2014) 20:1371–5. doi: 10.1016/j.parkreldis.2014.09.026
9. Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm.* (2003) 110:517–36. doi: 10.1007/s00702-002-0808-2
10. Klingelhoefer L, Reichmann H. Pathogenesis of Parkinson disease–the gut-brain axis and environmental factors. *Nat Rev Neurol.* (2015) 11:625–36. doi: 10.1038/nrneurol.2015.197
11. Lionnet A, Leclair-Visonneau L, Neunlist M, Murayama S, Takao M, Adler CH, et al. Does Parkinson's disease start in the gut? *Acta Neuropathol.* (2018) 135:1–12. doi: 10.1007/s00401-017-1777-8
12. Brundin P, Melki R. Prying into the prion hypothesis for Parkinson's disease. *J Neurosci.* (2017) 37:9808–18. doi: 10.1523/JNEUROSCI.1788-16.2017
13. Surmeier DJ, Obeso JA, Halliday GM. Parkinson's disease is not simply a prion disorder. *J Neurosci.* (2017) 37:9799–807. doi: 10.1523/JNEUROSCI.1787-16.2017
14. Rietdijk CD, Perez-Pardo P, Garssen J, van Wezel RJ, Kraneveld AD. Exploring Braak's hypothesis of Parkinson's disease. *Front Neurol.* (2017) 8:37. doi: 10.3389/fneur.2017.00037
15. Visanji NP, Brooks PL, Hazrati LN, Lang AE. The prion hypothesis in Parkinson's disease: braak to the future. *Acta Neuropathol Commun.* (2013) 1:2. doi: 10.1186/2051-5960-1-2
16. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
17. Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci Lett.* (2006) 396:67–72. doi: 10.1016/j.neulet.2005.11.012
18. Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. *Mov Disord.* (2012) 27:709–15. doi: 10.1002/mds.23838
19. Perez-Pardo P, Kliest T, Dodiya HB, Broersen LM, Garssen J, Keshavarzian A, et al. The gut-brain axis in Parkinson's disease: possibilities for food-based therapies. *Eur J Pharmacol.* (2017) 817:86–95. doi: 10.1016/j.ejphar.2017.05.042
20. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* (2007) 33:599–614. doi: 10.1111/j.1365-2990.2007.00874.x
21. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. *Ann N Y Acad Sci.* (2009) 1170:615–22. doi: 10.1111/j.1749-6632.2009.04365.x
22. Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T. Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977-2014. *Gut.* (2019) 68:18–24. doi: 10.1136/gutjnl-2017-315666
23. Ylonen S, Siitonen A, Nalls MA, Ylikotila P, Autere J, Eerola-Rautio J, et al. Genetic risk factors in Finnish patients with Parkinson's disease. *Parkinsonism Relat Disord.* (2017) 45:39–43. doi: 10.1016/j.parkreldis.2017.09.021
24. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* (2008) 7:583–90. doi: 10.1016/S1474-4422(08)70117-0
25. Hardy J. Genetic analysis of pathways to Parkinson disease. *Neuron.* (2010) 68:201–6. doi: 10.1016/j.neuron.2010.10.014
26. Liu Z, Lenardo MJ. The role of LRRK2 in inflammatory bowel disease. *Cell Res.* (2012) 22:1092–4. doi: 10.1038/cr.2012.42
27. Witoelar A, Jansen IE, Wang Y, Desikan RS, Gibbs JR, Blauwendraat C, et al. Genome-wide pleiotropy between Parkinson disease and autoimmune diseases. *JAMA Neurol.* (2017) 74:780–92. doi: 10.1001/jamaneurol.2017.0469
28. Kluss JH, Mamais A, Cookson MR. LRRK2 links genetic and sporadic Parkinson's disease. *Biochem Soc Trans.* (2019) 47:651–61. doi: 10.1042/BST20180462
29. Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet.* (2010) 42:1118–25. doi: 10.1038/ng.717
30. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet.* (2011) 43:246–52. doi: 10.1038/ng.764

31. McGovern DP, Gardet A, Torkvist L, Goyette P, Essers J, Taylor KD, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nat Genet.* (2010) 42:332–7. doi: 10.1038/ng.549
32. Sun M, He C, Cong Y, Liu Z. Regulatory immune cells in regulation of intestinal inflammatory response to microbiota. *Mucosal Immunol.* (2015) 8:969–78. doi: 10.1038/mi.2015.49
33. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord.* (2015) 30:350–8. doi: 10.1002/mds.26069
34. Yang D, Zhao D, Ali Shah SZ, Wu W, Lai M, Zhang X, et al. The role of the gut microbiota in the pathogenesis of Parkinson's disease. *Front Neurol.* (2019) 10:1155. doi: 10.3389/fneur.2019.01155
35. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell.* (2016) 167:1469–80 e12. doi: 10.1016/j.cell.2016.11.018
36. Gorecki AM, Preskey L, Bakeberg MC, Kenna JE, Gildenhuys C, MacDougall G, et al. Altered gut microbiome in parkinson's disease and the influence of lipopolysaccharide in a human alpha-synuclein over-expressing mouse model. *Front Neurosci.* (2019) 13:839. doi: 10.3389/fnins.2019.00839
37. McGee DJ, Lu XH, Disbrow EA. Stomaching the possibility of a pathogenic role for *Helicobacter pylori* in Parkinson's disease. *J Parkinsons Dis.* (2018) 8:367–74. doi: 10.3233/JPD-181327
38. Mridula KR, Borgohain R, Chandrasekhar Reddy V, Bandaru V, Suryaprabha T. Association of *Helicobacter pylori* with Parkinson's disease. *J Clin Neurol.* (2017) 13:181–6. doi: 10.3988/jcn.2017.13.2.181
39. Dardiotis E, Tsouris Z, Mentis AA, Siokas V, Michalopoulou A, Sokratous M, et al. *H. pylori* and Parkinson's disease: meta-analyses including clinical severity. *Clin Neurol Neurosurg.* (2018) 175:16–24. doi: 10.1016/j.clineuro.2018.09.039
40. Altschuler E. Gastric *Helicobacter pylori* infection as a cause of idiopathic Parkinson disease and non-arteric anterior optic ischemic neuropathy. *Med Hypotheses.* (1996) 47:413–4. doi: 10.1016/S0306-9877(96)90223-6
41. Shen X, Yang H, Wu Y, Zhang D, Jiang H. Meta-analysis: association of *Helicobacter pylori* infection with Parkinson's diseases. *Helicobacter.* (2017) 22. doi: 10.1111/hel.12398
42. Schulz JD, Hawkes EL, Shaw CA. Cycad toxins, *Helicobacter pylori* and parkinsonism: cholesterol glucosides as the common denomenator. *Med Hypotheses.* (2006) 66:1222–6. doi: 10.1016/j.mehy.2004.12.033
43. Wallen ZD, Appah M, Dean MN, Sesler CL, Factor SA, Molho E, et al. Characterizing dysbiosis of gut microbiome in PD: evidence for overabundance of opportunistic pathogens. *NPJ Parkinsons Dis.* (2020) 6:11. doi: 10.1038/s41531-020-0112-6
44. Burk K, Farecki ML, Lamprecht G, Roth G, Decker P, Weller M, et al. Neurological symptoms in patients with biopsy proven celiac disease. *Mov Disord.* (2009) 24:2358–62. doi: 10.1002/mds.22821
45. Di Lazzaro V, Capone F, Cammarota G, Di Giuda D, Ranieri F. Dramatic improvement of Parkinsonian symptoms after gluten-free diet introduction in a patient with silent celiac disease. *J Neurol.* (2014) 261:443–5. doi: 10.1007/s00415-014-7245-7
46. Garrett WS, Gordon JI, Glimcher LH. Homeostasis and inflammation in the intestine. *Cell.* (2010) 140:859–70. doi: 10.1016/j.cell.2010.01.023
47. Wershil BK, Furuta GT. 4. Gastrointestinal mucosal immunity. *J Allergy Clin Immunol.* (2008). 121(Suppl. 2):S380–3. doi: 10.1016/j.jaci.2007.10.023
48. Killinger B, Labrie V. The appendix in Parkinson's disease: from vestigial remnant to vital organ? *J Parkinsons Dis.* (2019) 9:S345–58. doi: 10.3233/JPD-191703
49. Clairembault T, Leclair-Visonneau L, Coron E, Bourreille A, Le Dily S, Vavasseur F, et al. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. *Acta Neuropathol Commun.* (2015) 3:12. doi: 10.1186/s40478-015-0196-0
50. Fang X. Impaired tissue barriers as potential therapeutic targets for Parkinson's disease and amyotrophic lateral sclerosis. *Metab Brain Dis.* (2018) 33:1031–43. doi: 10.1007/s11011-018-0239-x
51. Vidal PM, Pacheco R. Targeting the dopaminergic system in autoimmunity. *J Neuroimmune Pharmacol.* (2019) 15:57–73. doi: 10.1007/s11481-019-09834-5
52. Lee GR. The balance of Th17 versus treg cells in autoimmunity. *Int J Mol Sci.* (2018) 19:730. doi: 10.3390/ijms19030730
53. Stolzenberg E, Berry D, Yang, Lee EY, Kroemer A, Kaufman S, et al. A role for neuronal alpha-synuclein in gastrointestinal immunity. *J Innate Immun.* (2017) 9:456–63. doi: 10.1159/000477990
54. Gunnarsson LG, Bodin L. Occupational exposures and neurodegenerative diseases-a systematic literature review and meta-analyses. *Int J Environ Res Public Health.* (2019) 16:337. doi: 10.3390/ijerph16030337
55. Petrovitch H, Ross GW, Abbott RD, Sanderson WT, Sharp DS, Tanner CM, et al. Plantation work and risk of Parkinson disease in a population-based longitudinal study. *Arch Neurol.* (2002) 59:1787–92. doi: 10.1001/archneur.59.11.1787
56. Gao HM, Hong JS, Zhang W, Liu B. Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease. *J Neurosci.* (2003) 23:1228–36. doi: 10.1523/JNEUROSCI.23-04-01228.2003
57. Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. Rotenone, paraquat, and Parkinson's disease. *Environ Health Perspect.* (2011) 119:866–72. doi: 10.1289/ehp.1002839
58. Pan-Montojo F, Schwarz M, Winkler C, Arnhold M, O'Sullivan GA, Pal A, et al. Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. *Sci Rep.* (2012) 2:898. doi: 10.1038/srep00898
59. Purisai MG, McCormack AL, Cumine S, Li J, Isla MZ, Di Monte DA. Microglial activation as a priming event leading to paraquat-induced dopaminergic cell degeneration. *Neurobiol Dis.* (2007) 25:392–400. doi: 10.1016/j.nbd.2006.10.008
60. Cai Z, Fan LW, Kaizaki A, Tien LT, Ma T, Pang Y, et al. Neonatal systemic exposure to lipopolysaccharide enhances susceptibility of nigrostriatal dopaminergic neurons to rotenone neurotoxicity in later life. *Dev Neurosci.* (2013) 35:155–71. doi: 10.1159/000346156
61. Brudek T. Inflammatory Bowel diseases and Parkinson's disease. *J Parkinsons Dis.* (2019) 9:S331–44. doi: 10.3233/JPD-191729
62. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, et al. Pesticide exposure and risk for Parkinson's disease. *Ann Neurol.* (2006) 60:197–203. doi: 10.1002/ana.20904
63. Breckenridge CB, Berry C, Chang ET, Sielken RL Jr, Mandel JS. Association between Parkinson's disease and cigarette smoking, rural living, well-water consumption, farming and pesticide use: systematic review and meta-analysis. *PloS ONE.* (2016) 11:e0151841. doi: 10.1371/journal.pone.0151841
64. Aguirre-Mardones C, Iranzo A, Vilas D, Serradell M, Gaig C, Santamaria J, et al. Prevalence and timeline of nonmotor symptoms in idiopathic rapid eye movement sleep behavior disorder. *J Neurol.* (2015) 262:1568–78. doi: 10.1007/s00415-015-7742-3
65. Nihei Y, Takahashi K, Koto A, Mihara B, Morita Y, Isozumi K, et al. REM sleep behavior disorder in Japanese patients with Parkinson's disease: a multicenter study using the REM sleep behavior disorder screening questionnaire. *J Neurol.* (2012) 259:1606–12. doi: 10.1007/s00415-011-6386-1
66. Bazin T, Micoulaud Franchi JA, Terras N, Taillard J, Laharie D, Zerbib F, et al. Altered sleep quality is associated with Crohn's disease activity: an actimetry study. *Sleep Breath.* (2019) doi: 10.1007/s11325-019-01934-z
67. Sofia MA, Lipowska AM, Zmeter N, Perez E, Kavitt R, Rubin DT. Poor sleep quality in Crohn's disease is associated with disease activity and risk for hospitalization or surgery. *Inflamm Bowel Dis.* (2019) 26:1251–9. doi: 10.1093/ibd/izz258

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CORRECTION
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Corrigendum: Gut–Brain Axis: Potential Factors Involved in the Pathogenesis of Parkinson's Disease

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In the original article, there was an error. The findings cited in Reference Number 43 (Wallen et al., 2020) were inaccurately stated.

A correction has been made to *Common factors in the Pathogenesis of PD and Gastrointestinal Disorders, Gut Microbiota*, *Paragraph 3*. The corrected paragraph is shown below:

More recently, Wallen et al. conducted an association study (MWAS) between microbiome and PD using two large datasets. They found that the opportunistic pathogens and carbohydrate-metabolizing probiotics were significantly increased while short-chain fatty acid (SCFA)–producing bacteria were decreased in PD patients (43). These findings will facilitate testing the potential role of some of these pathogens in PD pathogenesis.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

REFERENCES

43. Wallen ZD, Appah M, Dean MN, Sesler CL, Factor SA, Molho E, et al. Characterizing dysbiosis of gut microbiome in PD: evidence for overabundance of opportunistic pathogens. NPJ Parkinsons Dis. (2020) 6:11. doi: 10.1038/s41531-020-0112-6

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