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RESEARCH ARTICLE

Cognitive and Alzheimer's disease biomarker effects of oral nicotinamide riboside (NR) supplementation in older adults with subjective cognitive decline and mild cognitive impairment

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Abstract

INTRODUCTION: Age-associated depletion in nicotinamide adenine dinucleotide (NAD+) concentrations has been implicated in metabolic, cardiovascular, and neurodegenerative disorders. Supplementation with NAD+ precursors, such as nicotinamide riboside (NR), offers a potential therapeutic avenue against neurodegenerative pathologies in aging, Alzheimer's disease, and related dementias. A crossover, double-blind, randomized placebo (PBO) controlled trial was conducted to test the safety and efficacy of 8 weeks' active treatment with NR (1 g/day) on cognition and plasma AD biomarkers in older adults with subjective cognitive decline and mild cognitive impairment.

METHODS: The primary efficacy outcome was the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Secondary outcomes included plasma phosphorylated tau 217 ($pTau^{217}$), glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL). Exploratory outcomes included Lumosity gameplay (*z*-scores) for cognition and step counts from wearables. Mixed model for repeated measures was used for between-group comparisons; paired *t*-tests were used for within-individual comparisons.

RESULTS: Forty-six participants aged over 55 were randomized to NR-PBO or PBO-NR groups; 41 completed baseline visits, and 37 completed the trial. NR supplementation was safe and well tolerated with no differences in adverse events reported between NR and PBO treatment phases. For the between-group comparison, there was a 7% reduction in $pTau^{217}$ concentrations after taking NR, while an 18% increase with PBO ($p = 0.02$). No significant between-group differences were observed for RBANS, other

plasma biomarkers(GFAP and NfL), Lumosity gameplay scores or step counts. For the within-individual comparison, pTau^{217} concentrations significantly decreased during the NR phase compared to the PBO ($p = 0.02$), while step counts significantly increased during the NR phase than PBO ($p = 0.04$).

DISCUSSION: Eight weeks NR supplementation is safe and lowered pTau^{217} concentrations but did not alter cognition as measured by conventional or novel digital assessments. Further research is warranted to validate NR's efficacy in altering pathological brain aging processes.

KEYWORDS
crossover design, phosphorylated tau pathologies, randomized controlled trial, treatment heterogeneity

Highlights

- The integrated study design combines a two-arm parallel trial with a crossover phase, offering the opportunity to enhance sample size for within-individual analysis and assess carryover effects.
- NR is safe but did not alter cognition as measured by multi-modal assessments in SCD/MCI.
- For between-group comparison, pTau^{217} levels decreased with NR and increased with PBO at 8-week follow-up.
- For within-individual comparison, step counts increased after NR and decreased after PBO.
- A larger, longer study with pharmacodynamic and pathophysiological biomarkers is needed to assess NR's disease-modifying effects.

1 | BACKGROUND

Cognitive impairment is common as people age, but it may not be a necessary part of normal aging.[1] Subjective cognitive decline (SCD) and mild cognitive impairment (MCI) can be transitional states between normal cognition and dementia. Although there is no universal consensus on their definitions, SCD generally refers to perceived cognitive changes despite normal performance on standardized cognitive tests,[2] whereas MCI involves both cognitive complaints and objective cognitive impairment without functional decline.[3] Individuals with SCD or MCI have increased risk of neurodegenerative dementia and may exhibit neuropathological alterations.[4–6]

Understanding the pathophysiology of Alzheimer's disease and related dementias (ADRDs) is critical for early disease modification. ADRDs are characterized by synaptic dysfunction and loss, neuronal cell death, and gliosis that predominantly affect cerebral association cortices and limbic subcortical structures.[7–9] The hallmark histopathological findings of AD include abnormal aggregates of extracellular amyloid plaques and intracellular tau neurofibrillary tangles.[10] Beyond these visible histopathologies, it is increasingly recognized that a host of pathophysiological processes contribute to neurodegeneration in aging and ADRD, including bioenergetic/metabolic dysfunction, endoplasmic reticulum stress, inflammation, and neurovascular injury.[11] These processes may interact with ADRD signature histopathologies in vicious cycles, presenting considerable challenges for comprehensive management, but also novel therapeutic targets.

Among many emerging therapeutic targets, nicotinamide adenine dinucleotide (NAD+) has received increasing attention due to its critical role in mitochondrial bioenergetics and cellular metabolism. NAD+ acts as the primary electron donor in the mitochondrial respiratory chain and regulates various enzymes involved in key metabolic pathways such as glycolysis, the Krebs cycle, and fatty acid oxidation.[12] NAD+ enables signaling for major cellular metabolism processes that often degrade with age, especially DNA repair mechanisms by activating sirtuins and serving as a substrate for poly(ADP-ribose) polymerase (PARP).[12] These mechanisms are critical for protection against neurodegeneration and play important roles in synaptic plasticity and neurotransmission. Studies in humans and animal models consistently show that intracellular NAD+ levels decline with age, as demonstrated in blood,[13] muscle,[14] saliva,[15] skin,[16] and brain.[17] This decline has been linked to metabolic and cardiovascular diseases,[18] frailty,[19] and neurodegenerative disorders.[20]

As a result, research groups are testing whether supplementation with NAD+ precursors, such as NR, nicotinamide mononucleotide

(NMN), or NAD+ itself,[21] could ameliorate neurodegenerative disorders, providing both disease modification and symptomatic benefit. In mouse models, replenishing NAD+ reverses age-related arterial dysfunction, oxidative stress, and metabolic dysfunction.[22–24] In AD mouse models, NR improves performance in behavioral tests, restores hippocampal synaptic plasticity, and attenuates Aβ or phosphorylated tau pathologies.[25–28] Early-phase human studies have shown that, NR treatment is well-tolerated in adults and older adults with MCI.[18,29–32] Airhart et al. conducted a non-randomized dose-escalation study on eight healthy adults, finding that 1 g of NR twice daily is well-tolerated and increased whole-blood NAD+ levels by 35%–168% above baseline by day 9.[30] Conze et al. assessed the kinetics and dose-dependency of NR chloride in overweight adults and found that blood NR exponentially declined from 3-12 h with a half-life of 2.7 h.[29] Doses of 100, 300, and 1000 mg NR chloride significantly increased blood NAD+ by 22%, 51%, and 142% within 2 weeks, respectively, with no significant adverse events (AEs). Orr et al. conducted a 10-week, placebo-controlled study on NR in older adults with MCI, and found that it increased blood NAD+ levels and was well-tolerated but did not improve cognition.[31] Despite one study examining the cognitive effects of NR, the uncertain impact of NR on AD plasma biomarkers demands investigation in a group at-risk for age-related NAD+ depletion. The current study tested the safety and effects of NR in cognition and ADRD biomarkers in older adults with SCD or MCI.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed existing literature on Alzheimer's disease (AD) pathophysiological pathways and potential benefits of nicotinamide riboside (NR) from animal models and human clinical trials. The effect of NR on cognitive impairment has not been widely studied. These relevant studies were cited.
2. **Interpretation**: Our findings were consistent with previous studies, suggesting that NR is safe but did not alter cognition in older adults with subjective cognitive decline/mild cognitive impairment (SCD/MCI) in a relatively short term. However, a potential NR effect in reducing pTau^{217} levels was observed, suggesting a potential therapeutic avenue for addressing neurodegeneration.
3. **Future directions**: A Phase II-III trial with larger sample size and longer duration of follow-up is warranted to investigate the effect of NR on pathological brain aging in the context of cognitive impairment.

2 | METHODS

2.1 | Study design, randomization, intervention

This trial was a double-blind, PBO-controlled randomized sequence block trial (NCT04078178) conducted at two sites: Massachusetts General Hospital (MGH, Boston, MA, USA) and Northern Light Acadia Hospital (Bangor, ME, USA). The randomization schedule was prepared by an independent statistician at MGH and implemented by the MGH research pharmacy for both sites. Participants were randomized 1:1 to the NR-PBO or PBO-NR sequence groups with age and sex a priori stratifications, but no other biomarkers. NR (TruNiagen) and matching encapsulated excipient PBO were provided by ChromaDex Inc. (Irvine, CA, USA).

The trial consisted of screening, a 4-week PBO lead-in period followed by two consecutive 8-week treatment periods (Blocks 1 and 2). After the initial visit of the lead-in period, all participants were provided PBO (two pills twice/day) for 4 weeks. After the PBO lead-in period, participants had a baseline assessment visit and were provided either NR (250 mg, two pills twice/day) or PBO (two pills twice/day) for 8 weeks based on their sequence assignment. A second assessment was conducted at the end of Block 1 after which NR or PBO was switched to PBO or NR in Block 2, with assessment at the end of 8 weeks. A brief check-in about safety was performed at baseline, crossover, and end-of-study (Figure 1).

The current integrated study design combines a conventional two-arm parallel design with an additional crossover phase. While this integrated design is more complex, it offers several advantages. In the two-arm parallel design framework, participants are randomly assigned to 8 weeks of NR orPBO to examine between-group changes. The added crossover phase after 8 weeks NR/PBO allows all participants to receive NR and PBO at different times. This approach doubles the sample size and increases the statistical power to detect within-individual changes. The crossover phase also enables the exploration of potential carryover effects by comparing the lead-in biomarker value with the end-of-study biomarker value in the NR-PBO group.

2.2 | Participants

Inclusion criteria included: (1) age 55 or older; (2) memory and other cognitive complaints consistent with SCD or MCI. We recognize that SCD and MCI exist on a continuum and discrete categorization is difficult. In this study, SCD was defined as self-reported perception of decline in cognitive performance in daily life compared to younger ages while still performing within the normal range on standardized cognitive measures.[33] MCI was defined as self-reported cognitive problems and performance below normative ranges in standardized cognitive measures, but without significant impairment in daily independent functioning.[34] Exclusion criteria included: (1) CNS disease history contributing to cognitive complaints other than suspected ADRD; (2) unstable medical or psychiatric illness; (3) hypersensitivity to NR or NMN; (4) no 30-day consumption of NR or NMN. Full-list criteria were provided (Table S1). Adjudication into SCD or MCI was based on the screening and baseline medical and functional assessment instruments by the clinician-investigators (neurologist/psychiatrist [S.E.A.], neuropsychologist [V.J.W. and N.S.], research nurse practitioner [A.J.M.]).

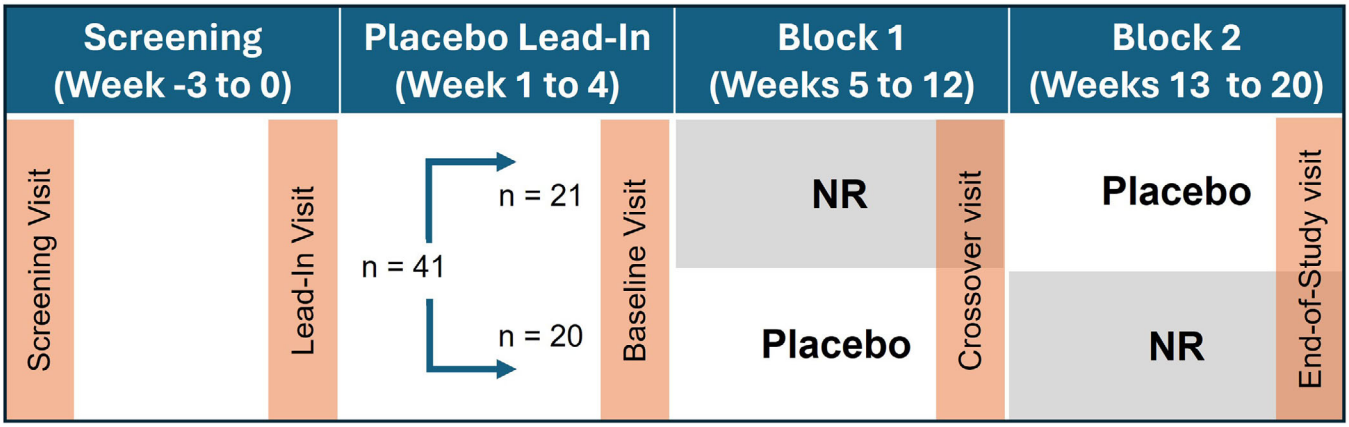

FIGURE 1 Integrated study design: two-arm parallel groups with a crossover phase.

We targeted 60 participants (30/group). A sample size of 26 per group was sufficiently powered to detect a Cohen's *d* of 0.8 ($\alpha = 0.05$) of between-group difference in an AD biomarker.

2.3 | Study end points

2.3.1 | Safety and compliance

Participants received boxes of medicine from study staff and discussed any questions about tolerability or AEs with a study team member at baseline, crossover, and end-of-study. Safety and tolerability assessed at lead-in, baseline, crossover, and end-of-study included vital signs and physical and neurological examinations. AEs were solicited at these visits and at telephone visits midway in each block. Compliance was assessed by pill counting for boxes of medicine returned at each visit.

2.3.2 | Cognitive function

The primary outcome was the total scaled score on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS).[35] Different versions of the RBANS were administered at baseline, crossover, and end-of-study. The RBANS total scaled score is a measure of global cognitive function with subdomains including attention, immediate memory, delayed memory, language, and visuospatial. The scaled score for total and subdomains ranges from 40 to 160, with a higher score indicating better cognitive performance. The RBANS possesses good psychometric properties and has been used as an outcome tool in clinical trials.[36] A previous study demonstrated that a lower RBANS total scaled score was associated with AD pathological biomarker positivity, reduced hippocampal volumes, increased cerebral β-amyloid, and the presence of the apolipoprotein E (APOE) ε4 allele.[37] Studies have shown that practice effect has diagnostic and prognostic utility, and an absence of practice effect has been linked to decline in MCI.[38] Therefore, we use the change score (crossover - baseline score) of RBANS as the outcome.

2.3.3 | ADRD plasma biomarkers

Phosphorylated tau 217 ($pTau^{217}$), glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL) were measured in plasma samples. Blood was drawn at lead-in, baseline, crossover, and end-of-study. The collection and processing of plasma sample was described in the Supporting Information. Percentage changes from baseline $pTau^{217}$, GFAP, and NfL concentrations were used for the analysis.

2.3.4 | Physical activitycStep count

Participants were provided with a Fitbit wearable device to monitor step counts, which they were required to wear continuously throughout the trial. Among the 36 participants with Fitbit data, 8 used the Fitbit Charge 3 (5 NR-PBO group; 3 PBO-NR group), while 28 used the Fitbit Charge 4 (14 in each group). Daily total step counts were calculated by summing the step counts for each day. The average daily step count over the last 14 days was used to assess walking intensity at baseline and during each block. Wear adherence was determined by the percentage of days with recorded step count data. The percentage change in step counts from baseline was used as the outcome, helping to normalize and center the data relative to the baseline.

2.3.5 | Lumosity brain health gameplay

Lumosity (Lumos Labs Inc., San Francisco, CA) is an online program consisting of games for brain health.[39] Participants were offered a tablet. Six out of 12 games (Table S2) were randomly assigned to participants when they logged in each day. Participants were instructed to play six unique Lumosity games at least once per day for 6 days/week throughout the trial, but participants were allowed to play as much as they liked. For each game, a z-score transformation was applied to the raw data of all participants over the entire trial period. The daily averages of these z-scores were then calculated to obtain the daily total score. A higher total score indicates better performance. For analysis, daily total

scores were extracted and averaged over the 2 weeks before baseline visits, the 2 weeks before crossover, and the 2 weeks before end-of-study to map with other assessments. The mean change in Lumosity scores from baseline to crossover was estimated to gauge individual learning effects and employed as an outcome in the subsequent analyses.

2.4 | Statistical analysis

Descriptive statistics were used to analyze between-group differences in demographic variables. Analysis of variance (ANOVA) was used to compare the compliance of pill-taking, wearable usage, and Lumosity gameplay between the two groups and across three study visits.

2.4.1 | Primary analysis

For all outcomes, we used intention-to-treat analysis and mixed-effects model repeated measures (MMRM) to examine between-group differences for the two-arm parallel design.[40] Outcomes included change scores from baseline in RBANS, Lumosity, and step counts, as well as percentage changes from baseline in plasma biomarkers. Group (PBO-NR vs. NR-PBO), time, and group*time were included as fixed effects. An unstructured covariance matrix was used to account for the correlation of repeated measures within a participant. We conducted four sensitivity analyses within MMRM: (1) age, education, and cognitive status were included as covariates. (2) The amount of gameplay was included as a covariate. (3) The model of Fitbit (Charge 3 vs. 4) was included as a covariate for the step count models. Since sensitivity analyses did not change the direction of findings, we reported the results without covariates.

2.4.2 | Secondary analysis

For within-individual comparisons in a crossover design, paired *t*-tests were used to compare biomarker concentrations between NR and PBO phases for all participants. SAS 9.4 (Cary, NC, USA) was used for all the analyses.

3 | RESULTS

3.1 | Study population

Participants were enrolled from September 2020 to August 2022. A total of 62 potential participants were screened for eligibility; 46 were randomized and 37 completed the trial (Figure 2). Twenty-one participants (51%) were assigned to follow the NR-PBO sequence, and 20 participants (49%) were assigned to follow PBO-NR sequence. NR-PBO and PBO-NR groups did not differ in baseline characteristics, except for education (Table 1).

3.2 | Compliance

The average pill-taking compliance rates were 94.22 ± 4.91% and 94.78 ± 7.16% in the NR-PBO and PBO-NR groups, respectively (Figure S1). There were no differences in the pill-taking compliance rate between two groups and across study visits (group*study; $p = 0.57$). Three participants exhibited a low compliance rate (<80%) during Block 2 (two in the NR-PBO; one in the PBO-NR).

3.3 | Safety and tolerability

The AEs that occurred during the trial were shown in Table 2. During the study, three serious AEs were reported. These events were determined to be unrelated/possibly related to the study drug. They included a urinary tract infection (lead-in phase), dizziness/transient ischemic attack (PBO phase), and an exacerbation of chronic underlying gait disorder (NR phase). In total, 57 AEs were reported throughout the study (lead-in: 23; NR phase: 15; PBO phase: 19). There were two AEs reported (post lumbar puncture headache; open sore from wearing Fitbit) that were considered related to the study during the lead-in period while participants were provided with the PBO. Safety laboratory results were provided (Table S3).

3.4 | Cognitive function

As for the primary analysis (between-group comparison during the first 8 weeks), there were no differences in the RBANS total scaled score ($p = 0.55$), nor for the five subdomains (Figure 3). There was a significant difference in RBANS attention scores, with the PBO-treated group demonstrating, on average, higher attention scores compared to the NR-treated group (difference: 5.07, $p = 0.04$). However, this finding became insignificant after controlling for age, education, and cognitive status. As for the secondary analysis (within-individual comparisons), no significant differences were observed for total and subdomain scores.

3.5 | ADRD plasma biomarkers

As for the primary analysis (between-group comparison during the first 8 weeks), there was a significant group difference in $pTau^{217}$ concentrations (Cohen's $d = 0.8$; $p = 0.02$) (Figure 4). The NR-PBO group experienced a 7% reduction in $pTau^{217}$ concentrations after taking NR, while the PBO-NR group showed an 18% increase in $pTau^{217}$ concentrations with PBO. To further substantiate the finding, we replaced baseline $pTau^{217}$ values with lead-in $pTau^{217}$ values and re-ran the analysis. The significant group difference at crossover in $pTau^{217}$ remained (Cohen's $d = 0.71$; $p = 0.04$). There were no group differences in GFAP ($p = 0.35$) or NfL ($p = 0.42$) (Figure 4).

As for the secondary analysis (within-individual comparisons), there was a reduction in $pTau^{217}$ during NR phase (3% reduction) compared

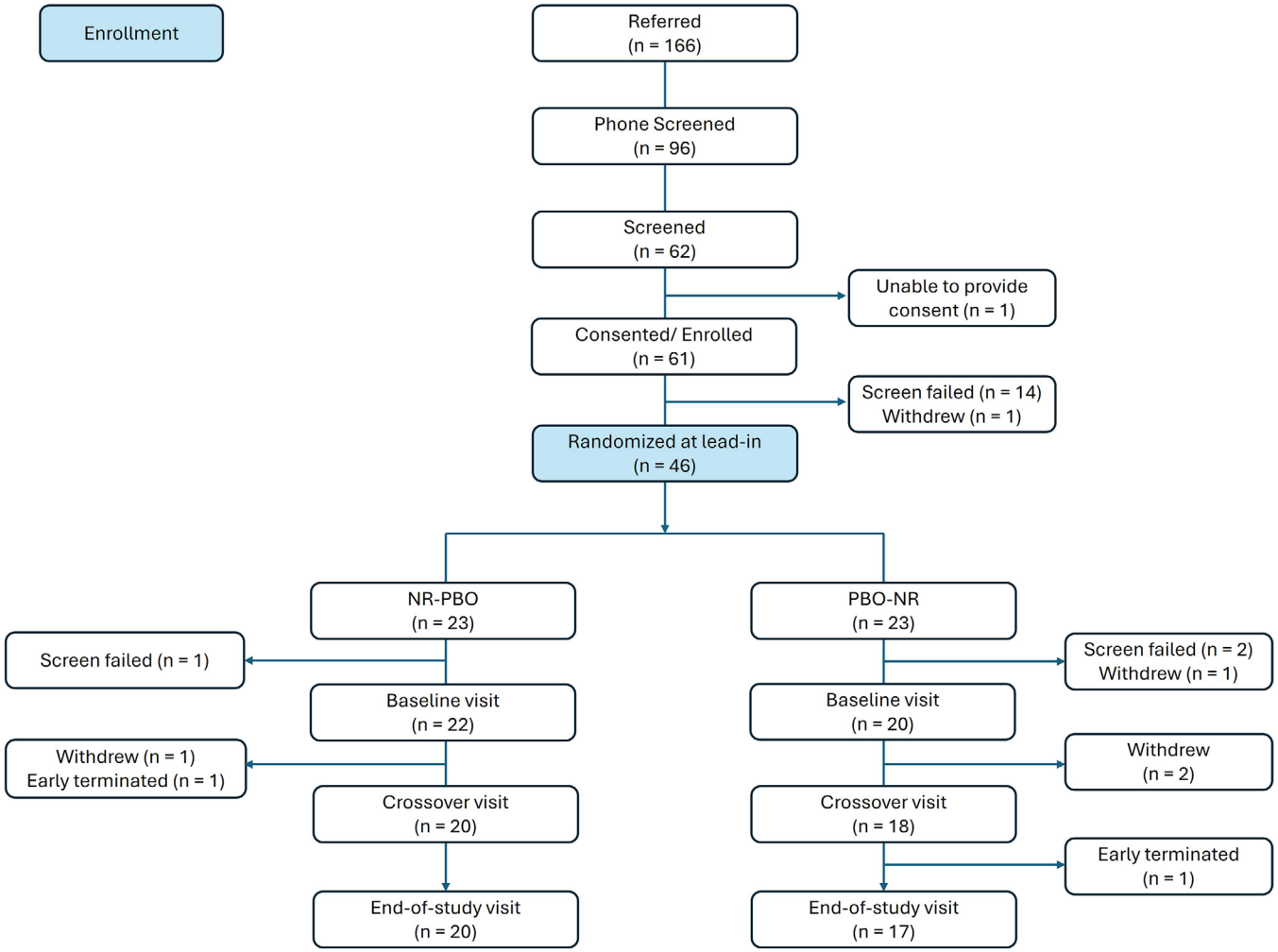

FIGURE 2 Consort diagram. In the NR-PBO group, one participant failed the screening due to medical safety concerns. In the PBO-NR group, two participants failed the screening: one due to medical safety concerns and another because the participant forgot to disclose prior usage of NR supplementation before the study began. NR, nicotinamide riboside; PBO, placebo

to PBO phase (17% increase) ($t = 2.35$, $p = 0.02$) (Figure 4). There were no between-phase differences in GFAP and NfL, respectively ($t = 1.70$, $p = 0.10$; $t = 1.17$, $p = 0.27$). The concentrations of plasma biomarker were provided (Table S4).

To investigate the carryover effect of NR on plasma biomarkers, we compared biomarker values at the lead-in and end-of-study in the NR-PBO group. No significant results were found for $pTau^{217}$ ($p = 0.48$) and NfL ($p = 0.90$). However, higher GFAP concentrations were observed at the end-of-study compared to lead-in ($p = 0.01$), suggesting a null finding and indicating that the 8-week washout period was sufficient.

3.6 | Lumosity brain health gameplay

On average, participants played Lumosity games 73.88% of total enrolled days generating data from 705.51±443.53 game sessions during the trial (Figure S1). There were no differences in the percentage days played between two groups and across study events (group*study events; $p = 0.44$), nor for number of game sessions played between the two groups at crossover ($p = 0.59$) and end-of-study ($p = 0.53$).

In terms of NR effect, there was no between-group or within-individual differences in the total game scores (Figure 3; Figure S2).

3.7 | Physical activity - Step count

The percentage of days with step count data was 90.23 ± 12.85% for the NR-PBO group and 92.72 ± 10.74% for the PBO-NR group. There were no significant differences in the percentage of days with step count data between the two groups and across study events (group*study events; $p = 0.22$).

As for the primary analysis (between-group comparison during the first 8 weeks), no significant difference was observed ($p = 0.37$). However, in the secondary analysis that focused on within-individual changes, there was an 11% increase in step counts following the use of

TABLE 1 Demographic and clinical characteristics of the participants at baseline

Characteristics	Randomized sequence groups		
	NR-PBO (*n*=21)	PBO-NR (*n*=20)	*p*-value
Age (years) [mean ± SD]	68.8 ± 6.6	65.2 ± 7.8	0.12
Sex (female) [*n* (%)]	11 (52%)	11 (55%)	0.87
Race (Caucasian) [*n* (%)]	20 (95%)	17 (85%)	0.27
Education (at least college) [*n* (%)]	14 (67%)	19 (95%)	0.02
Cognitive status [*n* (%)]			
SCD	14 (67%)	14 (70%)	0.82
MCI	7 (23%)	6 (30%)	
RBANS (scaled score) [mean±SD]			
Total	92.1 ± 11.7	95.8 ± 12.3	0.34
Immediate memory	92.4 ± 12.3	97.0 ± 18.5	0.37
Delayed memory	88.1 ± 18.5	97.1 ± 17.0	0.12
Attention	102.9 ± 12.5	99.9 ± 11.2	0.43
Language	100.8 ± 9.6	98.7 ± 9.8	0.51
Visuospatial	88.0 ± 15.1	92.9 ± 15.0	0.31
Plasma biomarkers (pg/ml) [mean ± SD]			
$pTau^{217}$	5.13 ± 4.19	4.23 ± 4.23	0.50
GFAP	66.38 ± 32.11	51.73 ± 32.37	0.15
NfL	174.6 ± 101.6	136.0 ± 67.71	0.17
Lumosity (*z*-score) [mean±SD]			
Total composite score	−0.65 ± 0.66	−0.38 ± 0.58	0.18
Brain shift	−0.76 ± 0.68	−0.48 ± 0.75	0.22
Chalkboard challenge	−0.33 ± 0.77	−0.14 ± 0.62	0.40
Color match	−0.69 ± 0.57	−0.45 ± 0.68	0.23
Ebb and flow	−0.95 ± 0.91	−0.36 ± 0.83	0.04
Follow that frog	−0.78 ± 0.87	−0.18 ± 0.68	0.03
Lost in migration	−0.86 ± 0.95	−0.46 ± 0.87	0.18
Masterpiece	−0.70± 0.75	−0.55 ± 0.86	0.58
Memory matrix	−0.27 ± 0.92	−0.18 ± 0.66	0.71
Pinball recall	−0.62 ± 0.83	−0.42 ± 0.73	0.42
Playing koi	−0.55 ± 0.87	−0.46 ± 0.70	0.73
Spatial speed match	−0.81 ± 0.74	−0.44 ± 0.74	0.12
Speed match	−0.82 ± 0.91	−0.51 ± 0.69	0.23
Step counts (daily) [mean ± SD][a]	5799 ± 2654	6354 ± 3903	0.62

Abbreviations: GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NfL, neurofilament light chain; NR, nicotinamide riboside; PBO, placebo; $pTau^{217}$, phosphorylated tau 217; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SCD, subjective cognitive decline; SD, standard deviation.

[a]Sample sizes for NR-PBO and PBO-NR are 19 and 17, respectively.

NR, in contrast to a 4% decrease in step counts after using the placebo ($t = 2.07, p = 0.04$). The raw step counts were provided (Table S4).

4 | DISCUSSION

The current study evaluated the safety and efficacy of NR in older adults with SCD/MCI. We conducted a randomized, crossover PBO-controlled trial with multi-modal assessments, including RBANS, Lumosity gameplay at home, wearables, and ADRD plasma biomarkers. NR was safe but did not alter cognition in older adults with SCD/MCI. Our examination of ADRD plasma biomarkers revealed a reduction in $pTau^{217}$ concentrations after treatment with NR. This possible decrease in $pTau^{217}$ concentrations suggests the need for larger and longer studies to replicate and investigate mechanisms by which NR may play a role in altering molecular biomarkers of brain pathology.

TABLE 2 Safety – Adverse events

No. of participants ever reported	Total	NR-PBO (*n*=21)			PBO-NR (*n*=20)		
		Lead-in	NR	PBO	Lead-in	PBO	NR
Serious adverse event	3 (100%)	0	0	0	1	1	1
Unrelated	2 (67%)	0	0	0	1[a]	1[b]	0
Possibly related	1 (33%)	0	0	0	0	0	1[c]
Related	0 (0%)	0	0	0	0	0	0
Adverse event	57 (100%)	17	8	9	6	10	7
Unrelated	37 (65%)	7	6	7	5	9	3
Possibly related	18 (32%)	8	2	2	1	1	4
Related	2 (3%)	2[d]	0	0	0	0	0

Abbreviations: NR, nicotinamide riboside; PBO, placebo.
[a]Urinary tract infection.
[b]Dizziness/transient ischemic attack.
[c]Exacerbation of chronic underlying gait disorder.
[d]Post lumbar puncture headache; open sore from wearing Fitbit.

We did not observe between-group differences in either RBANS, or the more novel, densely sampled Lumosity measures, suggesting that 8 weeks treatment with NR did not provide additional cognitive benefits compared with PBO. The between-group analysis indicated an unexpectedly higher RBANS attention score in the PBO group compared to the NR group. However, our sensitivity analysis – which included covariates such as age, education, and cognitive status – resulted in no significant findings. This suggested that the results might be confounded by demographics or cognitive severity. Our findings were consistent with the results of a recently reported 10-week parallel group randomized controlled trial study involving 20 older adults with MCI.[31] Their NR dosage was set to increase by 250 mg/day each week until reaching 1g/day at 4 weeks. This target dose was the same dose we used (from the start) and increased blood NAD+ levels by 2.6-fold. Their results showed that the NR group had less decline in MoCA over the 10 weeks than the PBO group, although this was not significant. However, the NR group showed worse post-treatment gait speed performance compared to the placebo group. In our current study, we found an increase in step counts with NR and a decrease in step counts with placebo from within-individual analysis. Notably, our primary, between-group analysis did not find significant group differences in step counts. Therefore, the effect of NR on physical activity/mobility or energy requires careful evaluation in future studies.

We used a similar dosage and treatment duration (8 vs. 10 weeks) as Orr et al.[31] but employed an integrated design. However, crossover designs introduce the possibility of carryover effects, especially for interventions with potential disease-modifying properties like NR. In the NR-PBO group, there is an 8-week washout period from crossover to end-of-study. This allowed us to examine whether an 8-week washout was sufficient by comparing lead-in and end-of-study biomarker values in the NR-PBO group. The 8-week washout was predetermined based on the fast clearance of NR in the blood. Generally, oral NAD+ supplements are metabolized relatively quickly in the body, with studies suggesting that blood NR levels decline exponentially from 3 to 12 h, with a half-life of 2.7 h in healthy adults.[30]

Given the reported benefits of NAD+ supplementation in AD mouse models, our study explored the effects of NR on AD plasma biomarkers. We observed a significant difference in $pTau^{217}$ concentrations by 1.23-folds between the randomized groups after 8 weeks. Our analysis revealed no significant difference between lead-in and end-of-study $pTau^{217}$ concentrations in the NR-PBO group, indicating a lower likelihood of carryover effects with an 8-week washout period. In the literature, $pTau^{217}$ concentrations were on average 2- to 4.4-folds higher in patients in the clinical AD-spectrum (or MCI with amyloid β+) compared to cognitively unimpaired controls.[41–43] This suggests that 8 weeks of NR treatment might reduce the difference of $pTau^{217}$ concentrations between the normal and MCI by 39%–72% (e.g., 1-(1.23/2) = 39%). Our findings are comparable to those of a 6-week randomized, placebo-controlled crossover trial that investigated the effects of NR on AD markers in 22 healthy older adults. After 6 weeks of oral NR (1g/day), amyloid β42 was significantly reduced among 9 out of 18 treatment responders whose NAD+ levels were increased.[44] However, $pTau^{181}$ and total tau were unaffected. Additionally, responders exhibited a significant reduction in biomarkers of neurodegenerative pathology in plasma neuronal-origin enriched extracellular vesicles (NEV).[44] This reduction was particularly noteworthy for phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2) and phosphorylated c-Jun N-terminal kinase (pJNK), both of which are associated with neuronal survival and amyloid deposits.[45,46]

Daily variability in cognitive abilities (good and bad days) is a common complaint of older adults. Our adoption of high-frequency, game-monitoring assessments represents a shift towards a more nuanced evaluation method to capture this more objectively.[47] It is important to note that we analyzed Lumosity using the game scores provided by its original designers without access to the features of each game. This

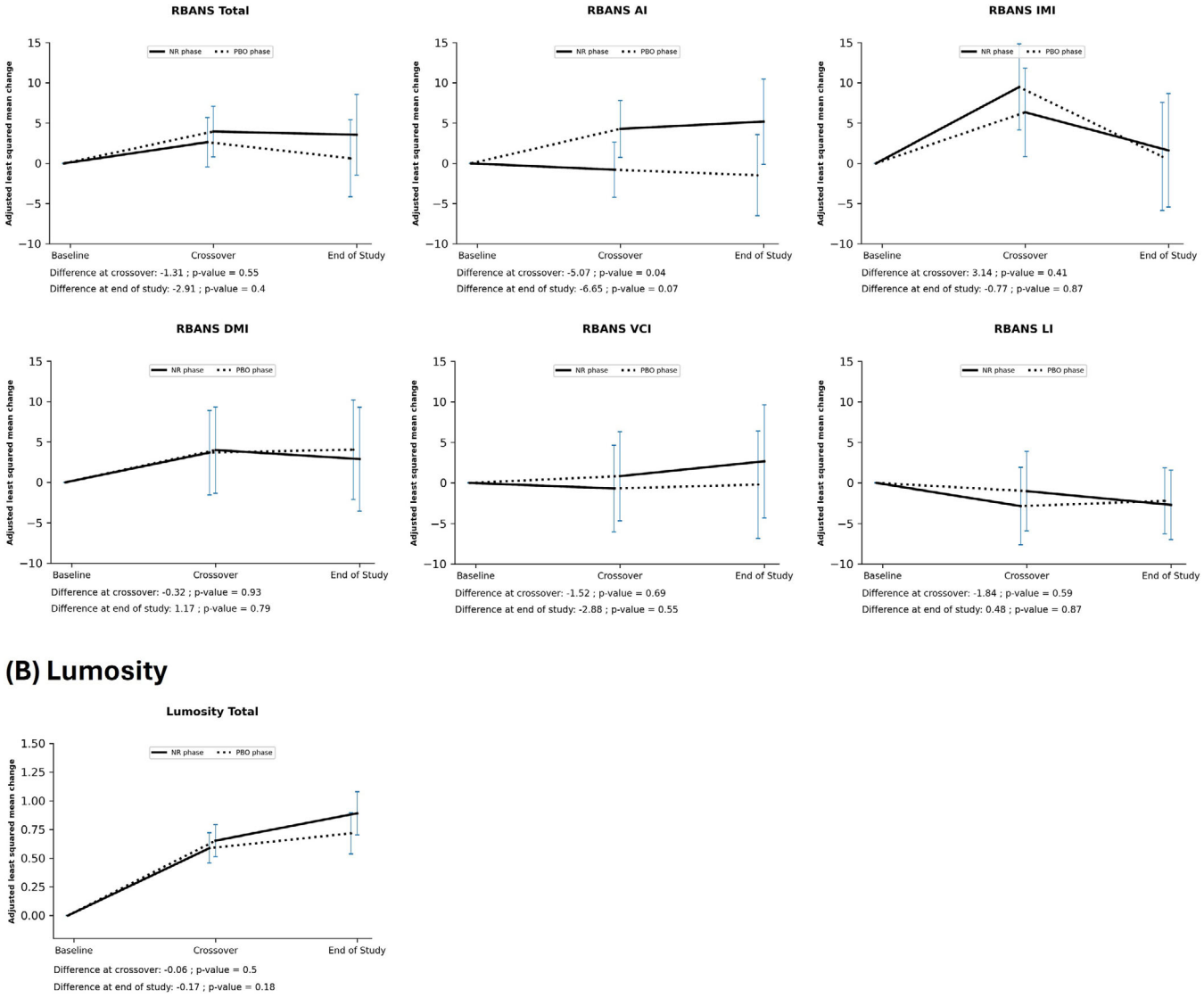

FIGURE 3 Mean change score of RBANS and Lumosity by the randomized groups. Adjusted mean change from baseline using MMRM stratified by randomized sequence groups for (A) RBANS and (B) Lumosity. AI, attention index; DMI, delayed memory index; IMI, immediate memory index; LI, language index; MMRM, mixed-effects model repeated measures; NR, nicotinamide riboside; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; VCI, visuospatial index

limitation hindered our ability to interpret scores meaningfully. Future trials could consider incorporating detailed game features to enhance the sensitivity of game-based monitoring measures.

4.1 | Study limitations and future directions

A few relatively small-scale and short-term NR trials, including this current investigation describe some promising results with NR,[31,44] but there remains a lack of evidence concerning the longer-term effects of NR on cognition in aging, neurodegeneration, and AD pathophysiology. The cognitive training and learning from Lumosity could potentially influence the overall findings and the RBANS may not have been sensitive to small cognitive changes. However, our sensitivity analysis, which included the amount of gameplay as a covariate, did not alter the overall direction of the results. However, the NR-PBO group in general had higher scores on Lumosity tasks than the PBO-NR group, suggesting that the two groups might not be well balanced in cognitive health. Our blood-based biomarker profiling of NR effects focused on three major ADRD biomarkers measured with highly qualified assays.[48] However, we did not measure in phosphatase/kinase activity, beta-amyloid 42/40 ratio, inflammatory, metabolic, or vascular biomarkers of relevance for aging and ADRD.[49] We also did not use standard instruments to assess safety, nor monitor blood NAD+ levels and other pharmacodynamic markers expected from NR supplement as a potential method for identifying those individuals more or less likely

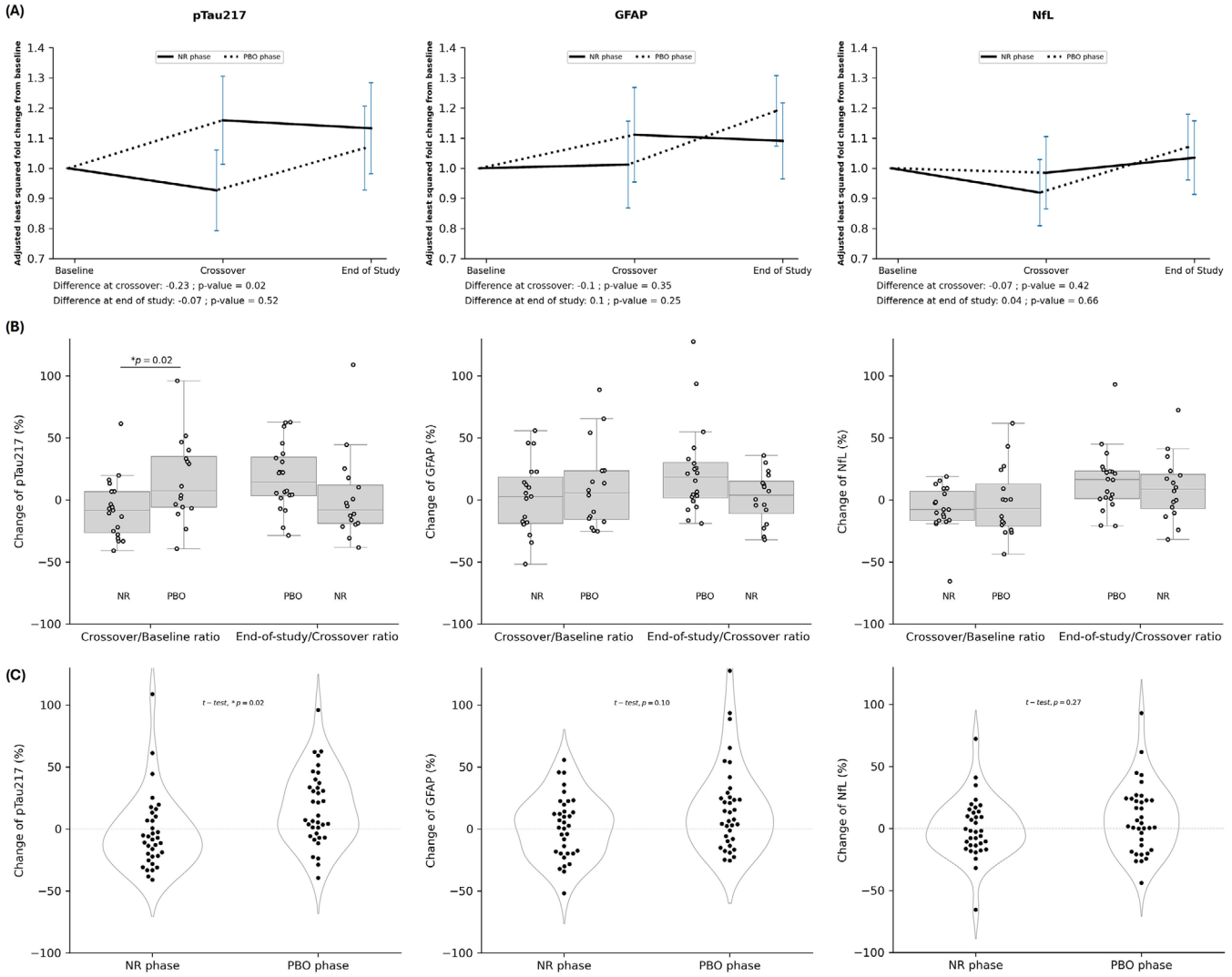

FIGURE 4 Change of ADRD plasma biomarkers concentrations by the randomized groups. (A) The line plots showed the MMRM results – adjusted least squared changes of plasma biomarkers from baseline (%). (B) The bar plots showed the changes of plasma biomarkers from (1) baseline to crossover and (2) from crossover to end-of-study. (C) The violin plots showed the distribution of changes at NR and PBO phases. Paired *t*-tests were used to assess whether there were significant differences in the changes of plasma biomarkers between phases. The gray horizontal line showed no within-individual changes (0%). The bar plots excluded two participants who only had baseline and end-of-study data. GFAP, glial fibrillary acidic protein; MMRM, mixed-effects model repeated measures; NfL, neurofilament light chain; NR, nicotinamide riboside; PBO, placebo; $pTau^{217}$, phosphorylated tau 217.

to respond. Future studies are needed to explore the heterogeneity of treatment responses of NR.

5 | CONCLUSIONS

During an 8-week period, the oral supplementation of 1g/day of NR was safe but did not alter cognition in older adults with SCD or MCI. Intriguingly, there was a potential effect of NR observed in terms of reducing $pTau^{217}$ concentrations. The diseases' progression may take several years before significant decline becomes apparent. Thus, a longer study with large diverse samples is necessary to explore the role of NR in potential disease-modifying effects.

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CONFLICT OF INTEREST STATEMENT

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Pharmaceuticals, Foster & Eldridge, LLP, Quince Therapeutics, Sage Therapeutics, and Vandria; received sponsored research grant support via his institution from the following commercial entities: AbbVie, Amylyx, Athira Pharma, Cyclerion Therapeutics, EIP Pharma, Ionis Pharmaceuticals, Janssen Pharmaceuticals, Inc., Novartis AG, Seer Biosciences, Inc. and vTv Therapeutics, Inc; and has received sponsored research grant support via his institution from the following non-commercial entities: Alzheimer's Association, Alzheimer's Drug Discovery Foundation, Challenger Foundation, Cure Alzheimer's Fund, John Sperling Foundation, the National Institutes of Health and the Prion Alliance. R.E.T. is a paid consultant for, and holds equity in Chromadex, Inc and was not involved with the execution of the trial. Author disclosures are available in the supporting information.

CONSENT STATEMENT

Study approval was obtained from the Institutional Review Board at Massachusetts General Brigham (MGB) (2019P002668) and all participants provided informed consent.

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SUPPORTING INFORMATION

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