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Effects of a Capsule Containing *Clostridium butyricum*, 3-(4-Hydroxy-3-methoxyphenyl) propionic Acid, and Salmon Milt-Derived DNA on Cognitive Function in Middle-Aged and Older Adults:

A 12-Week Randomized, Double-Blind, Placebo-Controlled Trial

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● **Summary**

Objective: To investigate whether a capsule containing *Clostridium butyricum*, 3-(4-hydroxy-3-methoxyphenyl) propionic acid (HMPA), and salmon milt-derived DNA improves cognitive function in middle-aged and older adults with subjective memory complaints.
Method: This 12-week randomized, double-blind, placebo-controlled trial enrolled 80 adults aged 55-79 years with subjective memory complaints and MMSE-J ≥ 24. Participants received Test food or Placebo once daily. The primary outcome was cognitive function assessed by Cognitrax. Secondary/exploratory outcomes included bowel habits, biochemical parameters, and safety. Efficacy was evaluated in the per-protocol set (n=74), and safety was evaluated in the full analysis set (n=80). An exploratory subgroup comprised participants aged ≥ 65 years with baseline MMSE-J scores of 24-27.
Results: Overall, no consistent between-group differences were observed in memory-related Cognitrax outcomes, but Symbol Digit Coding correct responses improved significantly in the Test food group. In the exploratory subgroup, Verbal Memory and Verbal Memory delayed correct hits improved significantly, whereas Symbol Digit Coding errors decreased significantly, versus Placebo. Bowel habit indices did not improve. Exploratory analyses suggested favorable changes in fasting glucose, HbA1c, gamma-glutamyl transferase (γ-GT), and uric acid. No serious adverse events or product-related side effects occurred.
Conclusion: The Test food did not show consistent overall memory-related benefit, but improved an attention-related index and, in a cognitively high-risk subgroup, improved verbal memory-related outcomes while an attention-related subtest score decreased. Together with unchanged bowel indices and favorable exploratory changes in selected metabolic markers, these findings suggest that metabolic changes may be involved, at least in part, in the observed cognitive effects.

Keywords: *Clostridium butyricum*; HMPA; salmon milt-derived DNA; cognitive function; gut-brain axis; randomized controlled trial

INTRODUCTION

The global burden of dementia and age-related cognitive decline continues to rise, underscoring the need for preventive strategies that can support brain health before the onset of overt dementia[1)-3)]. Among potential targets, the gut-brain axis has received increasing attention in nutrition and neuroscience. Bidirectional communication between the gut microbiota and the central nervous system is mediated by microbial metabolites, immune and endocrine pathways, and neural signaling, and gut dysbiosis has been linked to cognitive decline and neurodegenerative disorders[4)-8)]. In particular, reduced abundance of butyrate-producing bacteria has been reported in individuals with Alzheimer's disease and mild cognitive impairment (MCI), suggesting that impaired butyrate-related signaling may contribute to cognitive vulnerability[9)10)]. Because short-chain fatty acids, particularly butyrate, are involved in maintaining gut barrier integrity, regulating immune function, and modulating neuroinflammation, nutritional interventions targeting the gut microbiota may represent a practical approach to supporting cognitive function[5)].

The Test food evaluated in this study contained *Clostridium butyricum*, 3-(4-hydroxy-3-methoxyphenyl) propionic acid (HMPA) derived from fermented rice bran,

and salmon milt-derived DNA. *C. butyricum* has been reported to improve cognition-related outcomes in animal models through modulation of the microbiota-gut-brain axis, and butyrate-related interventions have shown neuroprotective effects and benefits for mitochondrial function[11)-14)]. HMPA and fermented rice bran have been associated with anti-amyloidogenic activity and cognitive benefits in older adults[15)16)], whereas salmon milt-derived DNA has been reported to exert memory-related and neuroprotective effects[17)18)]. In addition, previous studies have suggested that *C. butyricum*-containing interventions may improve the gut environment[19)20)]. In a previous study by our research group using food containing *C. butyricum* and HMPA, improvement in bowel habits was not evident in the overall population[21)], but a significant benefit was observed among participants with constipation tendencies[22)].

Beyond the gut-brain axis, emerging evidence suggests that metabolic dysfunction is also relevant to age-related cognitive decline. Metabolic abnormalities such as insulin resistance and impaired glucose metabolism have been associated with poorer cognitive performance and increased dementia risk[23)-25)]. In addition, probiotic interventions containing butyrate-producing bacteria have been reported to improve glycemic control[26)], and *C. butyricum* has shown beneficial effects on metabolic abnormalities, insulin resistance, and liver-related markers in preclinical studies[27)-30)]. HMPA has also been associated with improvements in glucose metabolism, lipid profiles, and visceral fat[31)-33)], while salmon milt-derived DNA has been reported to improve liver function-related indices[34)]. Together, these findings suggest that this multi-component food may influence cognitive outcomes through both gut-related and metabolic pathways. Accordingly, selected metabolic markers were also included as exploratory outcomes in this study.

Therefore, we conducted a 12-week randomized, double-blind, placebo-controlled trial in middle-aged and older adults with subjective memory complaints to evaluate the effects of this capsule on cognitive function. Bowel habit indices and selected metabolic markers were also assessed as secondary and exploratory outcomes, respectively, to examine possible pathways underlying any observed effects.

I SUBJECTS AND METHODS

1. Study design and ethics

This 12-week randomized, double-blind, placebo-controlled, parallel-group superiority trial with a 1:1 allocation ratio was conducted to evaluate the effects of the Test food on cognitive function and bowel habits. The study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Biological Research Involving Human Subjects in Japan. The study protocol was approved by the Ethics Review Committee of Chiyoda Paramedical Care Clinic (IRB No. 15000088; approval date: 21 March 2025). The study was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (TRN: UMIN000057405; URL: https://center6.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000065589; registration date: 26 March 2025) before initiation of the intervention. All participants provided written informed consent before enrollment. Participants were recruited between 26 March and 8 April 2025. Recruitment and management of study participants were conducted by CPCC Co., Ltd. (Tokyo, Japan), and the trial interventions and assessments were performed at Chiyoda Paramedical Care Clinic (Tokyo, Japan). The overall study period ran from 31 March to 5 December 2025. The full trial protocol and statistical analysis plan are available from the corresponding author upon reasonable request. This trial is reported in accordance with the CONSORT guidelines (**Appendix Table S1**).

2. Participants

Men and women aged 55–79 years were eligible if they had subjective memory complaints, had an MMSE-J score ≥ 24 at baseline, were able to understand the study procedures, and provided written informed consent. Participants were excluded if they habitually used foods, supplements, or medications that could affect the study outcomes and could not discontinue them during the study period; had diagnosed dementia; had markedly irregular dietary or lifestyle habits; participated in another clinical trial during the relevant period; had heavy alcohol consumption; had self-reported color blindness; had a history of or current serious disease of the brain, heart, liver, kidney, or gastrointestinal system; had allergy to medicines or foods, especially salmon; had recent blood donation or a planned cumulative blood sampling volume exceeding the allowable limit; or were otherwise judged inappropriate for participation by the investigators.

3. Sample size

Sample size was estimated using pilot data for the Cognitrax Composite Memory standardized score, which was used as the Cognitrax-derived index for trial planning. Assuming a two-sided significance level of 0.05 and 80% power, 58 participants were required. To allow for potential dropouts and protocol deviations during the 12-week intervention, the target sample size was set at 80 participants (40 per group).

4. Randomization, blinding, and intervention

After enrollment, participants were assigned in a 1:1 ratio to the Test food or Placebo group by an allocation manager from an independent third-party organization not directly involved in the study, using a computer-generated randomization procedure. Randomization was

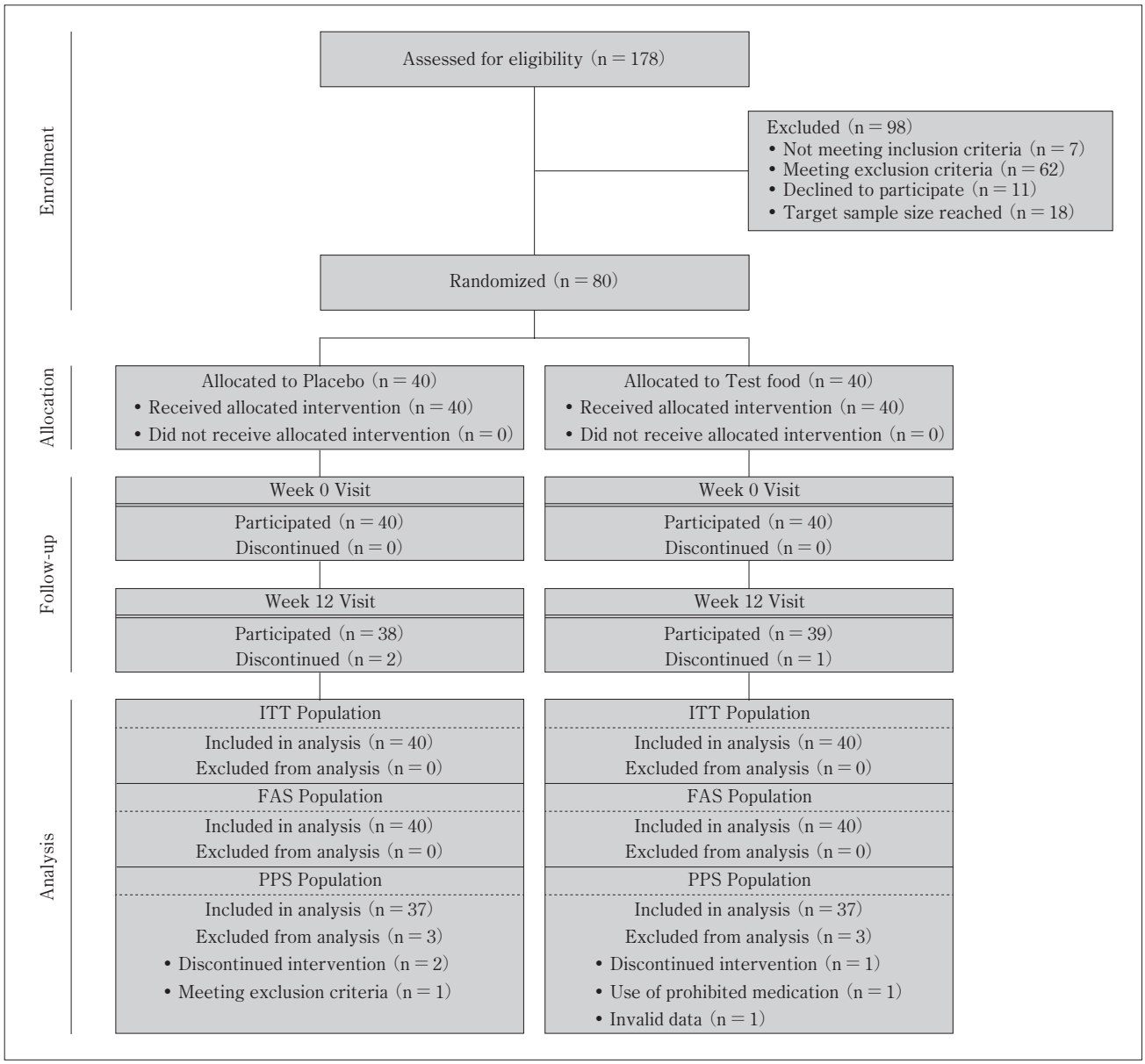


Fig. 1　CONSORT flow diagram.

balanced according to age, sex, Cognitrax Composite Memory standardized score, and daily defecation frequency during the 2-week pre-observation period. The Test food and Placebo capsules were indistinguishable in appearance and organoleptic characteristics. The study sponsor prepared and sealed the Study Product Identification Table, and the allocation list was separately managed by personnel not involved in study conduct. Both remained inaccessible to investigators and other study personnel until unblinding, thereby maintaining blinding throughout the trial.

The Test food was a capsule containing *C. butyricum* (1.4×10^7 CFU), fermented rice bran-derived HMPA (11.5 mg), and salmon milt-derived DNA (45 mg). Participants ingested one capsule daily with water or lukewarm water for 12 weeks. The Placebo capsule contained no active ingredients and was matched to the Test food in appearance and flavor. Detailed composition is shown in **Appendix Table S2**.

5. Outcomes

The primary outcome was cognitive function assessed using Cognitrax at baseline and week 12[35]. Prespecified analyses included standardized cognitive domain scores and subtest outcomes. Secondary outcomes were subjective cognitive function, mood, and bowel habits, and exploratory outcomes included selected metabolic markers and serum brain-derived neurotrophic factor (BDNF). Subjective cognitive function and mood were assessed at baseline and week 12 using a self-administered dementia checklist[36] and the Japanese short form of the Profile of Mood States 2 (POMS2)[37)-39)],

Table 1 Baseline characteristics of the participants.

Items（Unit）	Placebo (n = 37) Mean（SD）	Test food (n = 37) Mean（SD）	*p-value*
Age（years）	65.6（4.3）	65.6（4.1）	0.9265[a]
Male	24	23	1.0000[b]
Female	13	14	
Height（cm）	164.74（8.97）	164.66（7.94）	0.9684[c]
Body weight（kg）	63.23（11.61）	62.10（9.18）	0.6431[c]
BMI（kg/m^2）	23.15（2.89）	22.85（2.59）	0.8161[a]
MMSE-J score	27.4（1.9）	27.8（1.5）	0.3688[a]
Composite Memory（Cognitrax）	92.9（16.5）	96.1（19.9）	0.4481[c]
BDNF（pg/mL）	67.05（50.37）	330.85（968.43）	0.1520[a]
Defecation frequency（times/week）	7.9（3.9）	7.9（2.8）	0.7905[a]
Glucose（mg/dL）	96.0（8.2）	96.1（7.4）	0.9645[c]
HbA1c（NGSP, %）	5.50（0.28）	5.57（0.32）	0.2633[a]
Aspartate aminotransferase（U/L）	23.1（4.6）	22.5（5.9）	0.6456[c]
Alanine aminotransferase（U/L）	18.5（6.9）	19.6（8.3）	0.5190[c]
Gamma-glutamyl transferase（U/L）	26.2（12.4）	24.2（13.6）	0.3520[a]
Total cholesterol（mg/dL）	210.7（26.2）	206.7（25.9）	0.5110[c]
Triglycerides（mg/dL）	105.9（57.1）	93.5（37.5）	0.5377[a]
HDL cholesterol（mg/dL）	66.2（18.6）	66.7（15.3）	0.7661[a]
LDL cholesterol（mg/dL）	123.6（24.4）	122.9（27.4）	0.9146[c]

Values are presented as mean（SD）or n.

P values were calculated using the following tests: [a] Wilcoxon rank-sum test; [b] Fisher's exact test; [c] Student's t-test.

respectively. Bowel habits were recorded daily during the 2-week pre-observation period and the 12-week intervention period using participant diaries. Blood pressure, pulse rate, body weight, hematological and biochemical tests, urinalysis, and serum BDNF were evaluated at prespecified visits. Adherence was assessed from participant diaries and counts of unconsumed capsules, and adverse events were recorded throughout the study.

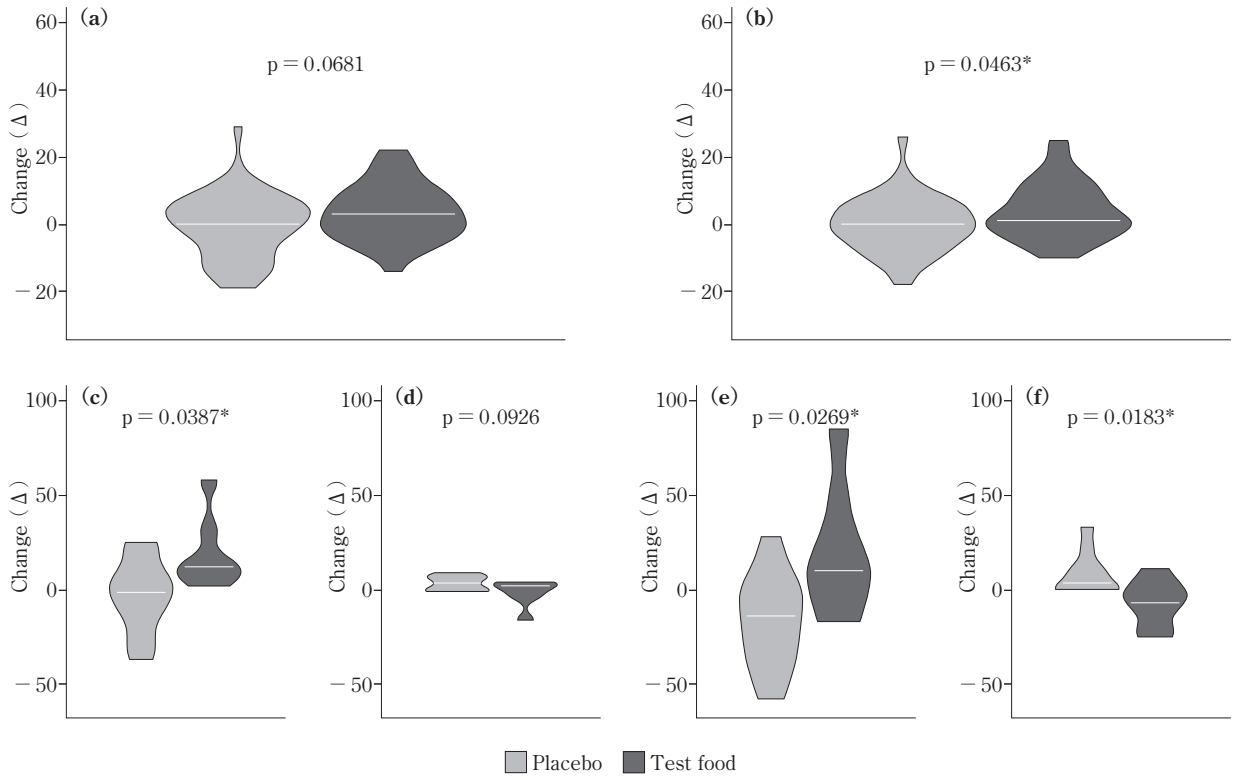
6. Statistical analysis

Statistical analyses were conducted according to a prespecified statistical analysis plan. Efficacy outcomes were analyzed in the per-protocol set, and safety was analyzed in the full analysis set. Missing values were not imputed. For efficacy outcomes, analyses were conducted using participants with complete data at all time points for each endpoint (complete-case analysis). For serum BDNF, when results were outside the assay's quantification range (below the lower limit of quantification [LLOQ] or above the upper limit of quantification [ULOQ]), the reference values reported by the laboratory were used for analysis. Between-group comparisons were primarily based on changes from baseline to week 12. Continuous variables were assessed for normality using the Shapiro–Wilk test. If normality was confirmed, Student's t-test or Welch's t-test was used for between-group comparisons, as appropriate; otherwise, the Wilcoxon rank-sum test was used. Paired t-tests or Wilcoxon signed-rank tests were used for within-group comparisons, as appropriate. Ordinal variables were analyzed using the Wilcoxon rank-sum test, and categorical variables, including adverse event incidence, were analyzed using Fisher's exact test. Bonferroni correction was applied to within-group comparisons of bowel indices. All tests were two-sided, with $p < 0.05$ considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 30.0 (IBM Corp., Armonk, NY, USA). An exploratory subgroup analysis conducted after database lock in participants aged ≥ 65 years with baseline MMSE-J scores of 24–27, as well as the related visualizations, was performed using R version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

II RESULTS

1. Participant flow and analysis sets

The participant flow is shown in **Fig. 1.** Of 178 individuals who provided written informed consent, 80 were enrolled and randomized to the Test food or Placebo group (40 per group). Three participants discontinued the study, and 77 completed the intervention. The full analysis set comprised all 80 randomized participants, and the per-protocol set comprised 74 participants (37 per group). Reasons for discontinuation and exclusions from the per-protocol set are shown in **Appendix Tables S3** and **S4**.



Changes from baseline (Δ) after 12 weeks of intervention are shown for selected Cognitrax outcomes in the per-protocol set. Panels a–b show the overall analysis: a, Processing Speed; b, Symbol Digit Coding (SDC) correct responses. Panels c–f show the exploratory subgroup analysis in participants aged ≥ 65 years with baseline MMSE-J scores of 24–27: c, Verbal Memory (VBM); d, Reaction Time; e, VBM delayed correct hits (subtest); and f, SDC errors (subtest). Δ indicates the change from baseline (week 12 minus baseline). Horizontal lines indicate the median. Between-group comparisons were performed using changes from baseline. *$p < 0.05$.

Fig. 2　Changes in selected Cognitrax outcomes in the overall and exploratory subgroup analyses.

2. Baseline characteristics

Baseline characteristics are shown in **Table 1**. No significant between-group differences were observed at baseline in age, sex, anthropometric measurements, cognitive function indices, bowel habits, or metabolic markers.

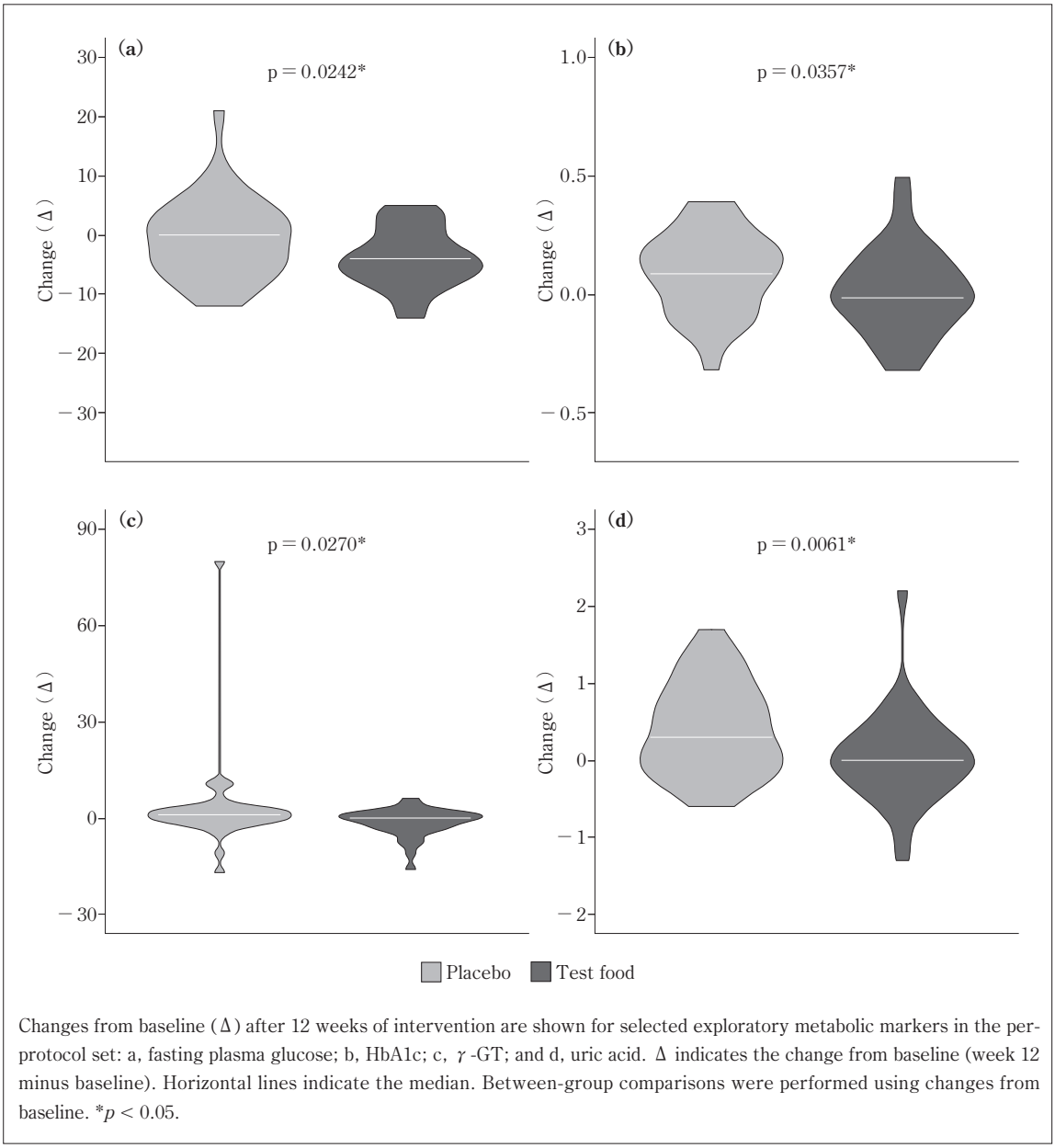
3. Cognitive function assessed by Cognitrax

Cognitive function assessed by Cognitrax was evaluated in the per-protocol set. Among the prespecified Cognitrax outcomes in the overall analysis, the standardized score for correct responses in the Symbol Digit Coding (SDC) subtest significantly improved in the Test food group compared with the Placebo group. Processing Speed also changed in a favorable direction, although the between-group difference did not reach statistical significance. No consistent between-group differences were observed across the remaining Cognitrax outcomes, including Composite Memory. Selected overall results are shown in **Fig. 2**, and full results are provided in **Appendix Table S5**.

In an exploratory subgroup analysis conducted after database lock in participants aged ≥ 65 years with baseline MMSE-J scores of 24–27, 17 participants were included (Placebo, n = 10; Test food, n = 7). In this subgroup, the standardized score for Verbal Memory (VBM) significantly increased in the Test food group compared with the Placebo group. In addition, VBM delayed correct hits increased and SDC errors decreased in the Test food group, while Reaction Time also decreased numerically without reaching statistical significance. Selected subgroup results are shown in **Fig. 2**, and full results are provided in **Appendix Table S6**.

4. Exploratory metabolic markers and related biochemical parameters

Exploratory analyses suggested favorable between-group differences in selected metabolic markers. Compared with the Placebo group, the Test food group showed lower changes in fasting plasma glucose, HbA1c, gamma-glutamyl transferase (γ-GT), and uric acid. These selected results are shown in **Fig. 3**, and full results are provided in **Appendix Table S7**. No clear between-group differences were observed in serum BDNF or in the other



Changes from baseline (Δ) after 12 weeks of intervention are shown for selected exploratory metabolic markers in the per-protocol set: a, fasting plasma glucose; b, HbA1c; c, γ-GT; and d, uric acid. Δ indicates the change from baseline (week 12 minus baseline). Horizontal lines indicate the median. Between-group comparisons were performed using changes from baseline. *$p < 0.05$.

Fig. 3 Changes in selected exploratory metabolic markers after 12 weeks of intervention.

biochemical parameters evaluated.

5. Other secondary outcomes

No consistent between-group differences were observed in bowel habit indices, subjective cognitive function, or mood. Although one subjective cognitive item showed a difference at week 12, no consistent between-group differences were observed across the overall subjective cognitive outcomes.

6. Compliance and safety

Treatment adherence was high in both groups. No serious adverse events were reported. Adverse events occurred in 12/40 participants (30%) in the Placebo group and 10/40 participants (25%) in the Test food group, with no significant between-group difference. No product-related side effects or clinically problematic changes attributable to study product intake were observed.

Ⅲ DISCUSSION

This 12-week randomized, double-blind, placebo-controlled trial evaluated a capsule containing *C. butyricum*, HMPA, and salmon milt-derived DNA in middle-aged and older adults with subjective memory complaints. In the overall analysis, the Test food group showed a significantly greater improvement in Symbol Digit Coding (SDC) correct responses, and Processing Speed changed in a favorable direction. In an exploratory subgroup of participants aged ≥ 65 years with baseline

MMSE-J scores of 24–27, verbal memory-related indices, including Verbal Memory (VBM) and VBM delayed correct hits, improved in the Test food group, whereas attention-related indices showed mixed changes, with a decrease in SDC errors and a numerical decrease in Reaction Time. No consistent between-group differences were observed in bowel habit indices, whereas exploratory analyses suggested favorable changes in selected metabolic markers. Taken together, these findings suggest that the Test food may exert domain-specific effects on cognitive function and that such effects may be more detectable in individuals at higher risk of cognitive decline.

The pattern of cognitive findings may also be clinically meaningful. In the overall population, the observed signal was mainly related to attention and information-processing efficiency, whereas in the exploratory high-risk subgroup it was more evident in verbal memory-related outcomes. Because deficits in verbal memory are among the cognitive changes most strongly associated with progression from mild cognitive impairment to Alzheimer's disease, these subgroup findings may be relevant from a preventive perspective[40] In addition, delaying the onset of dementia even by one year may substantially reduce the future global burden of dementia[3] At the same time, because the subgroup analysis was exploratory and involved a limited number of participants, these findings should be interpreted cautiously.

Previous studies support the biological plausibility of these findings. *C. butyricum* and butyrate-related interventions have been associated with neuroprotective and cognition-related effects through modulation of the microbiota-gut-brain axis and suppression of neuroinflammation[11)-14)], while HMPA and fermented rice bran-related interventions have been linked to anti-amyloidogenic activity and cognitive benefits[15)16)]. Salmon milt-derived DNA has also been reported to exert memory-related and neuroprotective effects[17)18)]. Together, these reports suggest that the three components may act through partially complementary pathways rather than through a single mechanism, although this study was not designed to determine the contribution of each component individually.

At the same time, the present findings provide limited support for a bowel-mediated gut-brain-axis effect as captured by bowel habit indices. No consistent between-group differences were observed in defecation frequency, stool characteristics, or related bowel symptoms. One plausible explanation is that constipation tendency was not part of the inclusion criteria and many participants appeared to have relatively stable bowel conditions at baseline. In such a population, improvement in the gut environment may not be readily reflected in bowel habit indices. This interpretation is also consistent with our previous study using food containing *C. butyricum* and HMPA, in which overall bowel improvement was not evident in the full population, whereas a significant benefit was observed among participants with constipation tendencies[21)22)]. Therefore, although a gut-brain-axis contribution cannot be excluded, the present study may not have been well suited to detect it using bowel-related outcomes alone.

In contrast, the improvements observed in selected metabolic markers may be more informative for interpreting the cognitive findings in this study. Compared with the Placebo group, the Test food group showed favorable changes in fasting plasma glucose, HbA1c, γ-GT, and uric acid. Although these changes were modest and exploratory, they suggest improvements in glucose metabolism and liver-related metabolic status. Previous studies have reported that probiotic interventions containing butyrate-producing bacteria can improve glycemic control[26)], and preclinical studies of *C. butyricum* have shown beneficial effects on metabolic abnormalities, insulin resistance, and liver-related indices[27)-30)]. HMPA has also been associated with improvements in glucose metabolism, lipid profiles, and visceral fat[31)-33)], whereas salmon milt-derived DNA has been linked to hepatoprotective effects[34)]. These findings raise the possibility that the Test food influenced cognitive function, at least in part, through improvements in systemic metabolic status.

Emerging evidence suggests that metabolic dysfunction is relevant to age-related cognitive decline. Metabolic abnormalities such as insulin resistance and impaired glucose metabolism have been associated with poorer cognitive performance and increased dementia risk[23)-25)]. Accordingly, the parallel observation of cognitive improvement in selected domains and favorable changes in metabolic markers in the present study raises the possibility that the Test food affected cognitive function, at least in part, through a metabolic-brain-axis pathway. In other words, the present results may be more compatible with a mechanism mediated by systemic metabolic improvement than with one primarily captured through bowel habit improvement. This interpretation should remain cautious, because the observed associations do not establish causality and mechanistic biomarkers directly linking metabolic change to neural function were not measured. Nevertheless, given the absence of clear bowel effects and the presence of favorable metabolic changes, the metabolic-brain-axis may provide a more plausible explanatory framework for the present findings.

The domain-specific pattern observed in this study may also be relevant when interpreting the subgroup findings. Memory function is closely related to medial temporal lobe structures, particularly the hippocampus[41)42)], and verbal episodic memory has been linked to structural and functional characteristics of the hippocampus[43)44)]. In contrast, information-processing speed and reaction time

have been associated with frontal lobe and frontal-subcortical circuits, and declines in these functions have been linked to frontal degeneration and white matter changes[45)46)]. The present pattern, in which verbal memory-related outcomes improved in the high-risk subgroup whereas attention-related indices showed mixed changes, may therefore be consistent with prior observations. However, because neurophysiological measures or neuroimaging were not collected, any interpretation regarding underlying neural substrates should remain exploratory.

LIMITATIONS

This study has several limitations. First, the sample size was modest and may have limited the power to detect between-group differences across multiple cognitive outcomes. Second, except for bowel indices, adjustment for multiple comparisons was not applied; therefore, significant findings across multiple cognitive and biochemical endpoints should be interpreted cautiously. Third, cognitive outcomes were evaluated over only 12 weeks, and longer-term studies are needed to determine the durability and clinical relevance of the observed effects. Fourth, the subgroup analysis was exploratory and conducted after database lock. Fifth, although favorable changes were observed in selected metabolic markers, these findings do not establish a causal relationship with cognitive outcomes. Finally, the present study did not include detailed mechanistic assessments, such as gut microbiota profiling, inflammatory markers, insulin resistance indices, or neuroimaging measures. Future studies in larger populations, with longer follow-up and in participants with bowel dysfunction or dysbiosis, are warranted to clarify the mechanisms underlying the observed effects.

CONCLUSION

In conclusion, 12-week intake of a capsule containing *C. butyricum*, HMPA, and salmon milt-derived DNA was associated with improvement in an attention-related Cognitrax index in the overall population and with improvements in verbal memory-related outcomes in an exploratory subgroup of older adults at higher risk of cognitive decline. In contrast, no consistent improvement was observed in bowel habit indices, whereas exploratory analyses suggested favorable changes in selected metabolic markers. These findings suggest that the cognitive effects of the Test food may be related, at least in part, to metabolic changes rather than to changes captured by bowel-related indices alone. Further studies in larger populations, with longer follow-up and detailed mechanistic assessments, are needed to confirm these findings and clarify the underlying pathways.

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DATA AVAILABILITY

The datasets generated and analyzed during the current study and the statistical code are available from the corresponding author upon reasonable request, subject to ethical and privacy restrictions.

COI

Y.T. and M.I. are employees of NICORIO Co., Ltd. A.U. is an employee of Ortho Corporation. This study was conducted as a collaborative project between NICORIO Co., Ltd. and Ortho Corporation, and was funded by NICORIO Co., Ltd., with partial financial support from Ortho Corporation. Both companies were involved in the study design, data analysis, data interpretation, and manuscript preparation. S.E. is a medical doctor at Chiyoda Paramedical Care Clinic and was contracted for the conduct of the clinical trial. Recruitment and management of study participants were conducted by CPCC Co., Ltd., and interventions and assessments were performed at Chiyoda Paramedical Care Clinic. The article processing charge was funded by NICORIO Co., Ltd. The authors made the final decision to submit the manuscript for publication.

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Appendix Table S1 CONSORT 2025 checklist.（1）

Section/topic	No	CONSORT 2025 checklist item description	Reported on page no.
Title and abstract			
Title and structured abstract	1a	Identification as a randomised trial	1
	1b	Structured summary of the trial design, methods, results, and conclusions	1
Open science			
Trial registration	2	Name of trial registry, identifying number（with URL）and date of registration	3
Protocol and statistical analysis plan	3	Where the trial protocol and statistical analysis plan can be accessed	3
Data sharing	4	Where and how the individual de-identified participant data（including data dictionary）, statistical code and any other materials can be accessed	11
Funding and conflicts of interest	5a	Sources of funding and other support（eg, supply of drugs）, and role of funders in the design, conduct, analysis and reporting of the trial	11
	5b	Financial and other conflicts of interest of the manuscript authors	11
Introduction			
Background and rationale	6	Scientific background and rationale	2
Objectives	7	Specific objectives related to benefits and harms	2
Methods			
Patient and public involvement	8	Details of patient or public involvement in the design, conduct and reporting of the trial	N/A
Trial design	9	Description of trial design including type of trial（eg, parallel group, crossover）, allocation ratio, and framework（eg, superiority, equivalence, non-inferiority, exploratory）	2-3
Changes to trial protocol	10	Important changes to the trial after it commenced including any outcomes or analyses that were not prespecified, with reason	4
Trial setting	11	Settings（eg, community, hospital）and locations（eg, countries, sites）where the trial was conducted	3
Eligibility criteria	12a	Eligibility criteria for participants	3
	12b	If applicable, eligibility criteria for sites and for individuals delivering the interventions（eg, surgeons, physiotherapists）	N/A
Intervention and comparator	13	Intervention and comparator with sufficient details to allow replication. If relevant, where additional materials describing the intervention and comparator（eg, intervention manual）can be accessed	3-4
Outcomes	14	Prespecified primary and secondary outcomes, including the specific measurement variable（eg, systolic blood pressure）, analysis metric（eg, change from baseline, final value, time to event）, method of aggregation（eg, median, proportion）, and time point for each outcome	4
Harms	15	How harms were defined and assessed（eg, systematically, non-systematically）	4
Sample size	16a	How sample size was determined, including all assumptions supporting the sample size calculation	3
	16b	Explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	17a	Who generated the random allocation sequence and the method used	3
	17b	Type of randomisation and details of any restriction（eg, stratification, blocking and block size）	3

Citation: Hopewell S, Chan AW, Collins GS, Hróbjartsson A, Moher D, Schulz KF, et al. CONSORT 2025 Statement: updated guideline for reporting randomised trials. BMJ. 2025; 388:e081123. https://dx.doi.org/10.1136/bmj-2024-081123

*We strongly recommend reading this statement in conjunction with the CONSORT 2025 Explanation and Elaboration and/or the CONSORT 2025 Expanded Checklist for important clarifications on all the items. We also recommend reading relevant CONSORT extensions. See www.consort-spirit.org.

Appendix Table S1　CONSORT 2025 checklist.（2）

Section/topic	No	CONSORT 2025 checklist item description	Reported on page no.
Allocation concealment mechanism	18	Mechanism used to implement the random allocation sequence（eg, central computer/telephone; sequentially numbered, opaque, sealed containers）, describing any steps to conceal the sequence until interventions were assigned	3
Implementation	19	Whether the personnel who enrolled and those who assigned participants to the interventions had access to the random allocation sequence	3
Blinding	20a	Who was blinded after assignment to interventions（eg, participants, care providers, outcome assessors, data analysts）	3
	20b	If blinded, how blinding was achieved and description of the similarity of interventions	3-4
Statistical methods	21a	Statistical methods used to compare groups for primary and secondary outcomes, including harms	4
	21b	Definition of who is included in each analysis（eg, all randomised participants）, and in which group	4
	21c	How missing data were handled in the analysis	4
	21d	Methods for any additional analyses（eg, subgroup and sensitivity analyses）, distinguishing prespecified from post hoc	4
Results			
Participant flow, including flow diagram	22a	For each group, the numbers of participants who were randomly assigned, received intended intervention, and were analysed for the primary outcome	4-5
	22b	For each group, losses and exclusions after randomisation, together with reasons	4-5
Recruitment	23a	Dates defining the periods of recruitment and follow-up for outcomes of benefits and harms	3
	23b	If relevant, why the trial ended or was stopped	N/A
Intervention and comparator delivery	24a	Intervention and comparator as they were actually administered〔eg, where appropriate, who delivered the intervention/comparator, how participants adhered, whether they were delivered as intended（fidelity）〕	4, 9
	24b	Concomitant care received during the trial for each group	N/A
Baseline data	25	A table showing baseline demographic and clinical characteristics for each group	6（Table 1）
Numbers analysed, outcomes and estimation	26	For each primary and secondary outcome, by group: ● the number of participants included in the analysis ● the number of participants with available data at the outcome time point ● result for each group, and the estimated effect size and its precision（such as 95% confidence interval） ● for binary outcomes, presentation of both absolute and relative effect size	6-9; Figure 2; Figure 3; Appendix Tables S5-S7
Harms	27	All harms or unintended events in each group	9
Ancillary analyses	28	Any other analyses performed, including subgroup and sensitivity analyses, distinguishing pre-specified from post hoc	4, 7, 9-10
Discussion			
Interpretation	29	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	9-11
Limitations	30	Trial limitations, addressing sources of potential bias, imprecision, generalisability, and, if relevant, multiplicity of analyses	10

Citation: Hopewell S, Chan AW, Collins GS, Hróbjartsson A, Moher D, Schulz KF, et al. CONSORT 2025 Statement: updated guideline for reporting randomised trials. BMJ. 2025; 388:e081123. https://dx.doi.org/10.1136/bmj-2024-081123

*We strongly recommend reading this statement in conjunction with the CONSORT 2025 Explanation and Elaboration and/or the CONSORT 2025 Expanded Checklist for important clarifications on all the items. We also recommend reading relevant CONSORT extensions. See www.consort-spirit.org.

Appendix Table S2 Composition and nutritional content of the Test food and Placebo capsules（per capsule）.

A）Composition（mg per capsule）

Ingredient	Test food	Placebo
Salmon milt extract	64.09	—
Rice bran fermented product	55.08	—
Clostridium butyricum powder	18.36	—
Reduced maltose syrup	8.67	191.4
HPMC（capsule shell）	46	46
Cellulose	11.9	17.6
Calcium stearate	6.8	11
Stabilizer（HPMC）	5.1	—

“—” indicates not included.

The Test food contained *Clostridium butyricum* at 1.4×10^7 CFU, HMPA at 11.5 mg, and salmon milt-derived DNA at 45 mg per capsule.

B）Nutritional content（per capsule; calculated values）

Nutrient	Test food	Placebo
Energy（kcal）	0.81	1.08
Protein（g）	0.057	0
Fat（g）	0.012	0.0082
Carbohydrate（g）	0.138	0.25
Salt equivalent（g）	0.013	0.00026

Appendix Table S3 Reasons for study discontinuation.

Subject No.	Group	Time point of discontinuation	Primary reason for discontinuation	Decision
11	Placebo	Day 0（first intake day）	Nasal symptoms（nasal irritation/rhinorrhea）reported from the first intake day; symptoms worsened and affected work	Discontinued by investigator
77	Placebo	Week 12（final visit）	Missed the final visit（Week 12）; rescheduling was not feasible within the site’s available dates	Discontinued（lost to follow-up at final visit）
85	Test food	During intervention, before Week 12	Newly diagnosed breast cancer; participant was withdrawn to prioritize medical treatment	Discontinued by investigator

Test food indicates the group receiving the Test food; Placebo indicates the group receiving the Placebo.
Day 0 refers to the first day of intake; Week 12 refers to the scheduled final assessment visit.

Appendix Table S4 Exclusions from the per-protocol set and reasons.

Subject No.	Group	Reason for PPS exclusion（summary）	Applicable PPS exclusion criterion
11	Placebo	Discontinued study（see Appendix Table S3）	PPS-1, PPS-2
23	Test food	Cognitrax standardized score outside the prespecified valid range（0–190）	PPS-4
25	Test food	Concomitant gastrointestinal medication（Pansiron G）; potential impact on efficacy endpoints	PPS-4
61	Placebo	Post-trial interview revealed liver cancer diagnosis; met exclusion criteria	PPS-6
77	Placebo	Discontinued study（see Appendix Table S3）	PPS-1
85	Test food	Discontinued study（see Appendix Table S3）	PPS-1, PPS-2

This table lists participants who met the predefined exclusion criteria for the per-protocol set（PPS）and were excluded from the PPS analysis.
PPS, per-protocol set; Cognitrax, computerized neurocognitive test battery used in this study.
PPS exclusion criteria:
PPS-1）Discontinued the study
PPS-2）Intake compliance < 85%
PPS-3）Repeated protocol violations regarding instructed precautions
PPS-4）Issues compromising reliability/validity of test results
PPS-5）After study start, newly found not to meet inclusion criteria or to meet exclusion criteria at baseline
PPS-6）During the study, clearly met exclusion criteria or exhibited prohibited behaviors
PPS-7）Other clear reason for exclusion from analysis

Appendix Table S5　Cognitrax outcomes in the PPS（n = 74）.（1）

Variable	Group	Baseline Mean (SD)	Week 12 Mean (SD)	Δ Mean (SD)	Δ diff. Mean (95% CI)	*p-value*（Δ）
Domain scores（standardized scores）						
Neurocognitive Index（NCI）	P T	102.4（ 8.2） 103.1（ 9.1）	105.1（ 7.3） 105.1（ 8.1）	2.7（ 5.1）[a] 2.0（ 5.5）[a]	− 0.7（− 3.1, 1.8）	0.5861[b]
Composite Memory	P T	92.9（16.5） 96.1（19.9）	98.0（15.5） 99.2（18.6）	5.1（14.1）[a] 3.1（14.2）	− 2.0（− 8.6, 4.5）	0.5396[b]
Verbal Memory	P T	92.8（19.4） 96.5（20.1）	98.6（18.0） 102.0（18.9）	5.8（19.2） 5.5（18.6）	− 0.3（− 9.0, 8.4）	0.9462[b]
Visual Memory	P T	95.5（13.0） 97.1（18.5）	98.2（15.1） 96.8（17.5）	2.7（16.4） − 0.3（13.8）	− 2.9（− 10.0, 4.1）	0.4070[b]
Psychomotor Speed	P T	107.5（12.9） 103.2（12.3）	109.2（11.9） 106.4（11.0）	1.7（ 5.5） 3.1（ 9.1）[a]	1.4（− 2.1, 4.9）	0.4232[c]
Reaction Time	P T	99.8（ 9.4） 101.1（10.9）	102.9（10.3） 101.8（ 9.2）	3.2（ 5.8）[a] 0.8（11.5）	− 2.4（− 6.7, 1.8）	0.2617[c]
Complex Attention	P T	108.6（ 8.7） 109.8（ 8.5）	109.3（10.4） 110.6（ 6.7）	0.7（ 9.7） 0.8（ 7.3）	0.1（− 3.8, 4.1）	0.9567[b]
Cognitive Flexibility	P T	103.4（10.8） 105.1（10.1）	105.9（ 9.4） 107.4（ 8.7）	2.6（ 9.2） 2.3（ 6.1）[a]	− 0.2（− 3.9, 3.4）	0.8939[c]
Processing Speed	P T	113.4（10.4） 111.5（11.2）	112.9（13.1） 114.9（10.3）	− 0.5（ 9.8） 3.5（ 8.3）[a]	3.9（− 0.3, 8.1）	0.0681[b]
Executive Function	P T	102.9（11.1） 104.5（10.1）	105.8（ 8.3） 107.1（ 8.5）	2.9（ 9.0） 2.6（ 6.2）[a]	− 0.4（− 4.0, 3.2）	0.8339[c]
Simple Attention	P T	103.0（11.4） 103.8（ 9.5）	101.5（10.0） 104.0（ 7.0）	− 1.5（12.6） 0.2（10.2）	1.8（− 3.5, 7.1）	0.5107[b]
Motor Speed	P T	101.5（13.2） 97.0（14.0）	103.9（11.3） 98.9（10.8）	2.5（ 5.5）[a] 1.9（11.0）	− 0.6（− 4.6, 3.5）	0.7806[c]

Data are shown as mean（SD）. The PPS included 74 participants: P, n = 37; T, n = 37. P, Placebo; T, Test food.

Δ, change from baseline to Week 12; Δ diff., between-group difference in Δ, calculated as T minus P and presented as mean difference with 95% CI. *p-value*（Δ）, between-group *p-value* for Δ.

Superscript a indicates $p < 0.05$ vs baseline within group, by paired t-test. Superscripts b and c for *p-value*（Δ）indicate the between-group test used: b, Student's t-test; c, Welch's t-test.

VBM, verbal memory test; VIM, visual memory test; FTT, finger tapping test; SDC, symbol digit coding test; ST, Stroop test; SAT, shifting attention test; CPT, continuous performance test.

All Cognitrax variables are presented as standardized scores.

Appendix Table S5 Cognitrax outcomes in the PPS（n＝74）. (2)

Variable		Group	Baseline Mean (SD)	Week 12 Mean (SD)	Δ Mean (SD)	Δ diff. Mean (95% CI)	*p-value*（Δ）
Test scores (standardized scores)							
VBM	Correct hits (immediate)	P T	96.8（19.3） 98.0（18.5）	103.8（12.2） 105.2（12.1）	7.0（15.4）[a] 7.2（15.6）[a]	0.2（－7.0, 7.4）	0.9465[b]
	Correct passes (immediate)	P T	98.8（16.8） 104.4（11.5）	103.4（11.5） 105.3（ 9.9）	4.6（14.1） 0.9（12.1）	－3.7（－9.8, 2.4）	0.2294[b]
	Correct hits (delayed)	P T	93.8（22.6） 94.0（27.4）	93.0（28.3） 96.7（27.8）	－0.8（29.8） 2.6（28.4）	3.5（－10.0, 17.0）	0.6082[b]
	Correct passes (delayed)	P T	94.3（20.4） 99.4（13.2）	99.2（16.2） 100.6（13.1）	5.0（15.6） 1.2（13.3）	－3.8（－10.5, 3.0）	0.2691[b]
VIM	Correct hits (immediate)	P T	91.2（18.3） 88.5（27.4）	97.1（17.1） 93.9（20.8）	5.9（16.7）[a] 5.4（20.7）	－0.5（－9.2, 8.2）	0.9117[b]
	Correct passes (immediate)	P T	102.1（16.0） 105.0（17.0）	102.4（14.7） 104.4（13.0）	0.3（15.3） －0.6（16.9）	－0.9（－8.3, 6.6）	0.8182[b]
	Correct hits (delayed)	P T	93.0（17.6） 93.8（23.3）	95.5（19.9） 94.2（24.7）	2.5（18.2） 0.4（15.8）	－2.1（－10.0, 5.8）	0.5917[b]
	Correct passes (delayed)	P T	102.2（14.2） 103.4（17.4）	100.1（15.3） 99.5（18.2）	－2.1（15.4） －3.8（14.7）	－1.7（－8.7, 5.3）	0.6284[b]
FTT	Right taps, average	P T	102.8（14.7） 97.8（16.5）	106.3（10.8） 100.7（10.9）	3.5（ 7.7）[a] 2.8（13.4）	－0.7（－5.8, 4.4）	0.7918[c]
	Left taps, average	P T	100.2（13.1） 96.6（10.8）	100.9（12.1） 97.4（10.7）	0.7（ 5.2） 0.8（ 9.3）	0.1（－3.4, 3.6）	0.9631[c]
SDC	Correct responses	P T	113.3（10.7） 110.8（11.5）	113.3（13.2） 114.7（11.0）	－0.1（ 8.4） 3.9（ 8.4）[a]	3.9（0.1, 7.8）	0.0463[b]
	Errors	P T	104.2（11.6） 108.4（ 8.6）	100.9（19.3） 105.8（10.0）	－3.3（22.6） －2.5（11.0）	0.8（－7.5, 9.0）	0.8553[c]
ST	Simple reaction time	P T	90.6（12.3） 90.6（22.3）	94.5（14.5） 94.8（17.3）	3.9（ 9.7）[a] 4.1（28.2）	0.3（－9.6, 10.2）	0.9563[c]
	Complex reaction time, correct	P T	98.1（ 8.4） 99.6（16.1）	100.4（ 9.4） 99.6（ 8.9）	2.3（ 5.6）[a] 0.0（16.3）	－2.3（－7.9, 3.4）	0.4265[c]
	Stroop reaction time, correct	P T	101.6（10.4） 102.4（ 8.5）	104.9（10.7） 103.9（ 9.5）	3.3（ 8.7）[a] 1.5（ 9.0）	－1.8（－5.9, 2.3）	0.3802[b]
	Stroop commission errors	P T	103.8（ 6.5） 104.0（ 6.0）	99.8（22.5） 102.6（ 6.4）	－4.0（22.4） －1.3（ 7.2）	2.7（－5.0, 10.4）	0.4927[c]
SAT	Correct responses	P T	99.8（12.0） 101.5（11.0）	102.9（ 9.8） 104.8（10.0）	3.2（ 8.5）[a] 3.4（ 6.1）[a]	0.2（－3.3, 3.6）	0.9251[c]
	Errors	P T	107.4（ 9.0） 108.8（ 8.2）	109.9（ 6.1） 109.9（ 6.0）	2.5（ 9.2） 1.1（ 6.3）	－1.4（－5.1, 2.3）	0.4468[c]
	Reaction time, correct	P T	103.3（12.8） 104.1（12.4）	106.5（12.7） 109.4（11.9）	3.2（ 8.8）[a] 5.2（ 8.0）[a]	2.0（－1.9, 5.9）	0.3050[b]
CPT	Correct responses	P T	103.4（ 0.8） 101.4（ 7.0）	100.5（10.9） 100.5（ 9.9）	－2.8（11.0） －0.9（11.0）	2.0（－3.1, 7.1）	0.4434[b]
	Omission errors	P T	103.4（ 0.8） 101.4（ 7.0）	100.5（10.9） 100.5（ 9.9）	－2.8（11.0） －0.9（11.0）	2.0（－3.1, 7.1）	0.4434[b]
	Commission errors	P T	101.5（14.8） 103.8（11.5）	101.1（ 9.9） 104.5（ 7.2）	－0.4（13.7） 0.7（11.3）	1.1（－4.7, 6.9）	0.7127[b]
	Reaction time, correct	P T	91.1（11.4） 89.6（12.9）	94.5（10.5） 93.9（10.2）	3.4（ 6.3）[a] 4.3（10.6）[a]	0.9（－3.2, 4.9）	0.6712[c]

Data are shown as mean (SD). The PPS included 74 participants: P, n = 37; T, n = 37. P, Placebo; T, Test food.
Δ, change from baseline to Week 12; Δ diff., between-group difference in Δ, calculated as T minus P and presented as mean difference with 95% CI. *p-value* (Δ), between-group *p-value* for Δ.
Superscript a indicates $p < 0.05$ vs baseline within group, by paired t-test. Superscripts b and c for *p-value* (Δ) indicate the between-group test used: b, Student's t-test; c, Welch's t-test.
VBM, verbal memory test; VIM, visual memory test; FTT, finger tapping test; SDC, symbol digit coding test; ST, Stroop test; SAT, shifting attention test; CPT, continuous performance test.
All Cognitrax variables are presented as standardized scores.

Appendix Table S6　Cognitrax outcomes in the high-risk subgroup（n＝17）.（1）

Variable	Group	Baseline Mean（SD）	Week 12 Mean（SD）	Δ Mean（SD）	Δ diff. Mean（95% CI）	*p-value*（Δ）
Domain scores（standardized scores）						
Neurocognitive Index（NCI）	P T	100.0（ 5.5） 102.7（15.2）	102.8（ 5.4） 105.9（11.2）	2.8（ 4.4） 3.1（ 5.1）	0.3（− 4.6, 5.3）	0.8841[b]
Composite Memory	P T	96.9（15.7） 94.0（22.5）	95.4（14.0） 104.3（19.5）	− 1.5（15.0） 10.3（17.4）	11.8（− 5.0, 28.6）	0.1560[b]
Verbal Memory	P T	96.1（19.4） 90.7（18.7）	93.1（14.4） 109.7（15.7）	− 3.0（19.9） 19.0（19.5）[a]	22.0（1.3, 42.7）	0.0387[b]
Visual Memory	P T	99.6（12.2） 98.9（23.7）	99.7（18.1） 97.4（21.6）	0.1（19.0） − 1.4（17.4）	− 1.5（− 20.8, 17.8）	0.8681[b]
Psychomotor Speed	P T	98.7（ 9.1） 105.3（21.2）	103.0（ 7.2） 107.4（ 9.8）	4.3（ 3.0）[a] 2.1（11.9）	− 2.2（− 10.4, 6.1）	0.6540[c]
Reaction Time	P T	99.3（ 7.7） 103.3（ 9.4）	102.7（10.0） 101.9（11.5）	3.4（ 4.0）[a] − 1.4（ 7.1）	− 4.8（− 10.6, 0.9）	0.0926[b]
Complex Attention	P T	105.5（ 7.2） 107.7（13.8）	108.3（ 7.1） 109.3（ 8.7）	2.8（ 8.0） 1.6（ 9.2）	− 1.2（− 10.1, 7.7）	0.7731[b]
Cognitive Flexibility	P T	99.4（ 9.0） 104.3（17.1）	104.2（ 7.3） 106.4（12.4）	4.8（ 7.9） 2.1（ 8.9）	− 2.7（− 11.4, 6.1）	0.5279[b]
Processing Speed	P T	107.7（ 8.6） 114.7（10.0）	109.7（ 7.9） 112.3（ 8.3）	2.0（ 3.7） − 2.4（ 7.8）	− 4.4（− 10.4, 1.5）	0.1984[c]
Executive Function	P T	99.4（ 9.0） 103.6（17.1）	104.1（ 7.0） 106.0（12.2）	4.7（ 7.4） 2.4（ 9.4）	− 2.3（− 10.9, 6.4）	0.5837[b]
Simple Attention	P T	101.3（13.6） 107.9（ 3.3）	100.4（ 9.9） 107.9（ 3.3）	− 0.9（10.2） 0.0（ 5.8）	0.9（− 8.3, 10.1）	0.8368[b]
Motor Speed	P T	93.4（ 8.2） 97.0（23.7）	98.5（ 6.6） 101.1（ 9.8）	5.1（ 4.8）[a] 4.1（15.7）	− 1.0（− 12.1, 10.2）	0.8806[c]

Data are shown as mean（SD）. The high-risk subgroup comprised participants aged ≥ 65 years with baseline MMSE-J scores of 24-27. The subgroup included 17 participants: P, n = 10; T, n = 7. P, Placebo; T, Test food.

Δ, change from baseline to Week 12; Δ diff., between-group difference in Δ, calculated as T minus P and presented as mean difference with 95% CI. *p-value*（Δ）, between-group *p-value* for Δ.

Superscript a indicates $p < 0.05$ vs baseline within group, by paired t-test. Superscripts b and c for *p-value*（Δ）indicate the between-group test used: b, Student's t-test; c, Welch's t-test.

VBM, verbal memory test; VIM, visual memory test; FTT, finger tapping test; SDC, symbol digit coding test; ST, Stroop test; SAT, shifting attention test; CPT, continuous performance test.

All Cognitrax variables are presented as standardized scores.

Appendix Table S6 Cognitrax outcomes in the high-risk subgroup（n = 17）. (2)

Variable		Group	Baseline Mean (SD)	Week 12 Mean (SD)	Δ Mean (SD)	Δ diff. Mean (95% CI)	*p-value*（Δ）
Test scores（standardized scores）							
VBM	Correct hits (immediate)	P	93.7（22.0）	99.3（ 8.7）	5.6（23.3）	9.0（− 12.2, 30.1）	0.3806[b]
		T	93.3（25.2）	107.9（15.4）	14.6（14.3）[a]		
	Correct passes (immediate)	P	104.7（ 6.7）	106.2（ 6.2）	1.5（ 9.3）	3.6（− 5.0, 12.3）	0.3849[b]
		T	100.7（13.2）	105.9（ 9.7）	5.1（ 6.4）		
	Correct hits (delayed)	P	98.7（15.2）	82.4（26.3）	− 16.3（25.8）	36.0（4.7, 67.3）	0.0269[b]
		T	89.4（28.0）	109.1（18.7）	19.7（35.0）		
	Correct passes (delayed)	P	97.2（11.4）	105.0（11.6）	7.8（ 7.7）[a]	− 9.1（− 21.5, 3.4）	0.2055[c]
		T	100.1（13.5）	98.9（12.9）	− 1.3（16.2）		
VIM	Correct hits (immediate)	P	91.1（16.2）	96.4（16.9）	5.3（18.4）	− 8.2（− 29.5, 13.2）	0.4274[b]
		T	89.4（38.9）	86.6（35.5）	− 2.9（22.8）		
	Correct passes (immediate)	P	102.7（16.4）	103.3（20.5）	0.6（17.6）	4.1（− 15.1, 23.4）	0.6552[b]
		T	108.6（24.0）	113.3（10.3）	4.7（19.4）		
	Correct hits (delayed)	P	95.2（16.7）	99.5（ 9.9）	4.3（16.6）	− 4.9（− 20.9, 11.2）	0.5277[b]
		T	97.4（29.7）	96.9（32.5）	− 0.6（13.1）		
	Correct passes (delayed)	P	108.9（10.3）	99.5（12.6）	− 9.4（11.4）[a]	7.8（− 7.9, 23.5）	0.3052[b]
		T	97.4（28.8）	95.9（20.7）	− 1.6（19.1）		
FTT	Right taps, average	P	95.2（11.0）	102.0（ 6.4）	6.8（ 7.5）[a]	− 1.5（− 15.8, 12.8）	0.8504[c]
		T	97.3（28.0）	102.6（ 9.7）	5.3（19.5）		
	Left taps, average	P	92.6（11.9）	95.0（ 8.9）	2.4（ 5.8）	0.7（− 7.3, 8.8）	0.8472[b]
		T	96.4（16.7）	99.6（ 9.2）	3.1（ 9.9）		
SDC	Correct responses	P	107.3（ 8.3）	108.3（ 8.4）	1.0（ 3.6）	− 2.4（− 7.6, 2.7）	0.3315[b]
		T	114.0（10.5）	112.6（ 8.9）	− 1.4（ 6.4）		
	Errors	P	103.9（11.8）	112.3（ 3.4）	8.4（11.3）[a]	− 15.5（− 28.1, − 3.0）	0.0183[b]
		T	110.0（ 4.9）	102.9（13.6）	− 7.1（12.7）		
ST	Simple reaction time	P	89.1（11.3）	91.5（16.9）	2.4（ 6.6）	− 7.8（− 32.1, 16.5）	0.5860[c]
		T	92.1（14.7）	86.7（33.8）	− 5.4（35.7）		
	Complex reaction time, correct	P	97.6（ 7.7）	101.3（ 9.2）	3.7（ 5.2）	− 3.7（− 8.7, 1.3）	0.1346[b]
		T	100.3（ 7.8）	100.3（10.5）	0.0（ 4.0）		
	Stroop reaction time, correct	P	100.8（ 8.0）	103.7（ 9.6）	2.9（ 5.0）	− 4.6（− 12.1, 2.9）	0.2110[b]
		T	105.3（ 9.4）	103.6（11.0）	− 1.7（ 9.6）		
	Stroop commission errors	P	100.1（ 7.6）	97.0（11.5）	− 3.1（15.8）	− 0.9（− 14.4, 12.6）	0.8733[c]
		T	106.4（ 5.4）	102.4（ 8.2）	− 4.0（ 6.3）		
SAT	Correct responses	P	95.6（10.0）	101.0（ 8.3）	5.4（ 7.7）	− 3.1（− 11.3, 5.0）	0.4283[b]
		T	102.4（17.5）	104.7（13.8）	2.3（ 7.8）		
	Errors	P	105.4（ 7.3）	108.8（ 5.4）	3.4（ 7.9）	− 0.8（− 10.5, 8.8）	0.8570[b]
		T	105.1（14.4）	107.7（ 7.8）	2.6（10.8）		
	Reaction time, correct	P	97.5（12.4）	103.2（11.3）	5.7（13.3）	− 2.3（− 13.5, 9.0）	0.6276[c]
		T	108.6（16.3）	112.0（15.3）	3.4（ 4.6）		
CPT	Correct responses	P	103.6（ 1.0）	100.5（ 9.3）	− 3.1（ 9.8）	3.1（− 4.9, 11.1）	0.4204[b]
		T	103.9（ 1.1）	103.9（ 1.1）	0.0（ 0.0）		
	Omission errors	P	103.6（ 1.0）	100.5（ 9.3）	− 3.1（ 9.8）	3.1（− 4.9, 11.1）	0.4204[b]
		T	103.9（ 1.1）	103.9（ 1.1）	0.0（ 0.0）		
	Commission errors	P	98.8（18.0）	100.3（10.3）	1.5（10.2）	− 1.5（− 11.6, 8.6）	0.7552[b]
		T	107.6（ 5.0）	107.6（ 5.0）	0.0（ 8.7）		
	Reaction time, correct	P	89.0（16.1）	93.1（11.1）	4.1（ 7.9）	− 5.8（− 14.1, 2.4）	0.1540[b]
		T	94.3（13.2）	92.6（14.4）	− 1.7（ 7.8）		

Data are shown as mean（SD）. The high-risk subgroup comprised participants aged ≥ 65 years with baseline MMSE-J scores of 24-27. The subgroup included 17 participants: P, n = 10; T, n = 7. P, Placebo; T, Test food.

Δ, change from baseline to Week 12; Δ diff., between-group difference in Δ, calculated as T minus P and presented as mean difference with 95% CI. *p-value*（Δ）, between-group *p-value* for Δ.

Superscript a indicates $p < 0.05$ vs baseline within group, by paired t-test. Superscripts b and c for *p-value*（Δ）indicate the between-group test used: b, Student's t-test; c, Welch's t-test.

VBM, verbal memory test; VIM, visual memory test; FTT, finger tapping test; SDC, symbol digit coding test; ST, Stroop test; SAT, shifting attention test; CPT, continuous performance test.

All Cognitrax variables are presented as standardized scores.

Appendix Table S7　Blood biochemistry outcomes in the PPS（n = 74）

Variable	Group	Baseline Mean (SD)	Week 12 Mean (SD)	Δ Mean (SD)	Δ diff. Mean (95% CI)	*p-value*（Δ）
TP（g/dL）	P	7.19（0.40）	7.20（0.35）	0.00（0.29）	− 0.06（− 0.19, 0.07）	0.4267[c]
	T	7.07（0.27）	7.02（0.32）	− 0.06（0.28）		
Alb（g/dL）	P	4.33（0.23）	4.37（0.23）	0.05（0.21）	− 0.03（− 0.13, 0.08）	0.6429[c]
	T	4.40（0.23）	4.42（0.27）	0.02（0.23）		
T-Bil（mg/dL）	P	0.68（0.23）	0.75（0.36）	0.07（0.22）	0.02（− 0.12, 0.17）	0.6482[c]
	T	0.75（0.27）	0.84（0.47）	0.09（0.39）		
ALP（U/L）	P	71.8（19.8）	73.2（16.2）	1.4（10.9）	− 2.08（− 6.51, 2.35）	0.5443[c]
	T	71.4（21.0）	70.7（19.5）	− 0.7（ 8.0）		
LDH（U/L）	P	180.0（23.1）	189.6（32.6）	9.6（19.8）[a]	− 0.24（− 8.67, 8.18）	0.9543[d]
	T	174.8（30.3）	184.2（31.9）	9.4（16.4）[a]		
AST（U/L）	P	23.1（ 4.6）	23.1（ 4.5）	0.1（ 4.3）	− 0.49（− 2.86, 1.88）	0.6832[d]
	T	22.5（ 5.9）	22.1（ 5.0）	− 0.4（ 5.8）		
ALT（U/L）	P	18.5（ 6.9）	19.3（ 7.1）	0.8（ 4.5）	− 1.92（− 4.54, 0.70）	0.5256[c]
	T	19.6（ 8.3）	18.5（ 5.8）	− 1.1（ 6.6）		
γ-GT（U/L）	P	26.2（12.4）	29.0（20.8）	2.8（14.0）	− 4.14（− 8.98, 0.71）	0.0270[c]
	T	24.2（13.6）	22.8（12.3）	− 1.4（ 4.3）		
CK（U/L）	P	142.2（66.4）	130.1（52.9）	− 12.1（50.3）	15.54（− 10.48, 41.56）	0.3225[c]
	T	130.8（61.3）	134.3（70.9）	3.5（61.4）		
TC（mg/dL）	P	210.7（26.2）	204.1（28.8）	− 6.6（21.1）	− 0.65（− 9.54, 8.24）	0.8847[d]
	T	206.7（25.9）	199.5（23.7）	− 7.2（17.0）[a]		
TG（mg/dL）	P	105.9（57.1）	110.5（65.5）	4.6（52.2）	− 1.95（− 22.52, 18.63）	0.9569[c]
	T	93.5（37.5）	96.1（48.8）	2.6（34.5）		
HDL-C（mg/dL）	P	66.2（18.6）	64.8（17.6）	− 1.5（ 7.9）	0.22（− 3.20, 3.63）	0.7866[c]
	T	66.7（15.3）	65.5（15.7）	− 1.2（ 6.8）		
LDL-C（mg/dL）	P	123.6（24.4）	119.2（27.8）	− 4.4（18.8）	0.62（− 7.24, 8.48）	0.8751[d]
	T	122.9（27.4）	119.1（25.5）	− 3.8（14.9）		
BUN（mg/dL）	P	15.7（ 3.9）	15.9（ 4.2）	0.2（ 2.5）	− 0.96（− 2.09, 0.18）	0.0981[d]
	T	14.0（ 3.1）	13.3（ 2.8）	− 0.8（ 2.4）		
Cr（mg/dL）	P	0.85（0.15）	0.89（0.16）	0.05（0.08）[a]	− 0.03（− 0.06, 0.00）	0.0638[d]
	T	0.82（0.16）	0.83（0.16）	0.02（0.06）		
UA（mg/dL）	P	5.35（1.14）	5.75（1.38）	0.41（0.59）[a]	− 0.39（− 0.66, − 0.11）	0.0061[d]
	T	5.51（1.26）	5.53（1.30）	0.02（0.59）		
Na（mEq/L）	P	141.5（ 1.5）	140.3（ 1.7）	− 1.2（ 1.5）[b]	0.14（− 0.62, 0.89）	0.6393[c]
	T	141.8（ 1.6）	140.8（ 1.9）	− 1.0（ 1.7）[b]		
K（mEq/L）	P	4.24（0.25）	4.26（0.32）	0.02（0.31）	− 0.06（− 0.20, 0.08）	0.3740[d]
	T	4.19（0.33）	4.15（0.32）	− 0.04（0.29）		
Cl（mEq/L）	P	103.6（ 1.9）	103.2（ 2.1）	− 0.4（ 1.7）	0.51（− 0.37, 1.39）	0.2318[c]
	T	103.4（ 2.5）	103.5（ 2.4）	0.1（ 2.0）		
Ca（mg/dL）	P	9.15（0.32）	9.22（0.28）	0.07（0.32）	− 0.09（− 0.24, 0.05）	0.2113[d]
	T	9.19（0.31）	9.16（0.26）	− 0.03（0.33）		
Glucose（mg/dL）	P	96.0（ 8.2）	95.3（ 7.6）	− 0.7（ 6.7）	− 3.16（− 5.90, − 0.42）	0.0242[d]
	T	96.1（ 7.4）	92.2（ 7.8）	− 3.8（ 5.1）[a]		
HbA1c（NGSP, %）	P	5.50（0.28）	5.61（0.27）	0.11（0.17）[b]	− 0.08（− 0.16, 0.00）	0.0357[c]
	T	5.57（0.32）	5.59（0.27）	0.02（0.18）		

Data are shown as mean（SD）. The PPS included 74 participants: P, n = 37; T, n = 37. P, Placebo; T, Test food.
Δ, change from baseline to Week 12; Δ diff., between-group difference in Δ, calculated as T minus P and presented as mean difference with 95% CI. *p-value*（Δ）, between-group *p-value* for Δ.
Superscript a indicates $p < 0.05$ vs baseline within group, by paired t-test. Superscript b indicates $p < 0.05$ vs baseline within group, by Wilcoxon signed-rank test. Superscripts c and d for *p-value*（Δ）indicate the between-group test used: c, Wilcoxon rank-sum test; d, Student's t-test.
Abbreviations: TP, total protein; Alb, albumin; T-Bil, total bilirubin; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyl transferase; CK, creatine kinase; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; HbA1c, hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program.