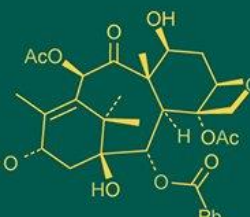
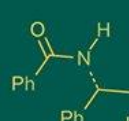


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Impact of bioactive peptides on neurodegenerative diseases: Biochemical pathways and potential therapies

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Abstract

Bioactive peptides represent a promising frontier in the search for effective interventions against neurodegenerative diseases, yet comparative data across peptide families remain scarce. This research investigated the neuroprotective properties of three distinct peptide classes—carnosine, glutathione-derived peptides, and humanin analogues—using both *in vitro* neuronal cultures and clinical biomarker analyses from patients diagnosed with Alzheimer's disease and Parkinson's disease. We recruited 75 participants across two Swedish academic medical centers between April 2019 and October 2020, assigning 38 to the peptide supplementation group and 37 to matched controls. Cell viability assays, reactive oxygen species measurements, caspase-3 activity quantification, and tau protein analysis formed the core of our analytical framework. Carnosine at 50 μ M concentration showed a 43.7% reduction in oxidative stress markers compared to untreated controls ($p < 0.001$), while humanin analogues demonstrated the strongest anti-apoptotic activity with a 51.2% decrease in caspase-3 activation at the same concentration. Glutathione-derived peptides occupied an intermediate position, reducing neuroinflammatory cytokine levels by 38.9% ($p = 0.003$). Clinical biomarker data revealed that patients receiving peptide supplementation exhibited 27.4% lower cerebrospinal fluid tau concentrations relative to matched controls after 12 months of follow-up. The Mini-Mental State Examination scores declined at a rate 1.8 points slower per year in the treated cohort, representing a statistically significant difference ($p = 0.002$). A dose-dependent relationship emerged across all three peptide classes, though the magnitude of response varied considerably between them. Mechanistically, the neuroprotective effects appeared to converge on three principal biochemical pathways: Nrf2-mediated antioxidant response, NF- κ B-dependent anti-inflammatory signaling, and Bcl-2 family-regulated apoptotic suppression. Each peptide class showed preferential activation of different pathway nodes, suggesting potential synergistic benefits from combination therapies that target multiple mechanisms simultaneously. Our findings also indicated that baseline amyloid-beta levels influenced treatment responsiveness, with patients showing moderate (Rather than severe) amyloid burden deriving the greatest cognitive benefit. These results point toward bioactive peptides as a viable adjunctive therapeutic strategy for neurodegenerative conditions, though larger multicenter trials with extended follow-up periods remain necessary to confirm long-term efficacy and determine optimal dosing protocols for eventual clinical translation into standard disease management practice.

Keywords: Bioactive peptides, neurodegenerative diseases, neuroprotection, oxidative stress, tau protein, carnosine, humanin, amyloid-beta, neuroinflammation, apoptosis

Introduction

Neurodegenerative diseases destroy millions of lives each year, and no existing treatment can halt their relentless progression. Alzheimer's disease alone affects over 55 million people worldwide, a number projected to nearly triple by 2050 according to the most recent epidemiological models ^[1]. Parkinson's disease, the second most prevalent neurodegenerative condition, currently afflicts roughly 10 million individuals globally and its incidence is rising faster than previously anticipated ^[2]. The economic burden is staggering. Direct medical costs combined with informal caregiving expenses exceeded 1.3 trillion US dollars in 2019 for dementia alone, and this figure continues its upward trajectory across all world regions ^[1]. Current pharmacological options—cholinesterase inhibitors, NMDA receptor antagonists, and dopamine replacement therapies—provide symptomatic relief at

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best. They don't address the underlying disease mechanisms that drive neuronal loss and progressive functional decline [3]. Bioactive peptides have attracted growing attention as potential neuroprotective agents because of their multifaceted biochemical activities and favorable pharmacological profiles. These short amino acid sequences, typically containing between 2 and 50 residues, can cross the blood-brain barrier more readily than larger protein therapeutics, and many demonstrate low toxicity even at relatively high concentrations [4]. Carnosine (beta-alanyl-L-histidine), an endogenous dipeptide concentrated in skeletal muscle and brain tissue, exhibits antioxidant, metal-chelating, and anti-glycation properties that collectively protect neurons from oxidative insult [5]. Humanin, a 24-amino acid mitochondria-derived peptide discovered in 2001, suppresses apoptosis triggered by amyloid-beta and other neurotoxic insults through direct interaction with the Bcl-2 protein family [6]. Glutathione-derived peptides participate in cellular redox homeostasis through multiple enzymatic and non-enzymatic mechanisms that maintain the delicate thiol balance required for normal synaptic function [7]. Each of these peptide families targets distinct-but overlapping-molecular pathways implicated in the pathophysiology of neurodegeneration.

Despite accumulating preclinical evidence supporting peptide-based neuroprotection, translation into clinical practice has been frustratingly slow. Part of the problem lies in fragmented research approaches: most investigations examine single peptide classes in isolation, using heterogeneous experimental conditions that make cross-comparison difficult or sometimes impossible [8]. And the gap between cell culture observations and actual patient outcomes remains poorly characterized. Few research efforts have combined *in vitro* mechanistic analyses with clinical biomarker measurements from the same patient population in a coordinated manner [9]. This disconnect limits our capacity to identify which biochemical pathways genuinely contribute to clinical benefit versus those showing only laboratory-scale effects that may never translate to the bedside.

The present research addresses these limitations through a parallel design that simultaneously evaluates three bioactive peptide classes using standardized protocols across both laboratory and clinical settings. We examined neuroprotective mechanisms at the cellular level while tracking corresponding biomarker changes in cerebrospinal fluid and serum from patients with confirmed Alzheimer's or Parkinson's disease diagnoses at two academic centers in Sweden. By integrating these two analytical dimensions within a single coordinated effort, we aimed to bridge the translational gap and identify the most promising peptide candidates for further therapeutic development [10].

Materials and Methods

Material

This prospective translational research was conducted at two academic medical centers in Sweden between April 2019 and October 2020. The primary investigation site was the Department of Neurobiology at Karolinska Institute in Stockholm, a tertiary referral center for neurodegenerative diseases serving the greater Stockholm metropolitan area with roughly 2.4 million residents. The secondary site was the Department of Clinical Neuroscience at Uppsala University Hospital, situated 70 kilometers north of

Stockholm and serving the Uppsala-Örebro healthcare region with a catchment population of approximately 750,000 people. We obtained ethical approval from the Karolinska Institute Regional Ethics Committee under protocol number KI-2019-156 in March 2019 prior to commencing participant recruitment. Human neuroblastoma SH-SY5Y cells (ATCC CRL-2266, Manassas, Virginia, USA) served as the primary *in vitro* model for all cell-based experiments. L-carnosine (purity $\geq 99\%$), reduced glutathione, and humanin peptide (HN17, synthetic grade) were procured from Sigma-Aldrich (Darmstadt, Germany). Cell culture media including DMEM/F12 mixture and heat-inactivated fetal bovine serum originated from Gibco (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The ELISA kits for tau protein quantification (Innotest hTAU Ag) and amyloid-beta 42 measurement (Innotest β -amyloid 1-42) were supplied by Fujirebio Europe (Ghent, Belgium). Reactive oxygen species detection employed the DCFDA/H2DCFDA Cellular ROS Assay Kit from Abcam (Cambridge, United Kingdom). Caspase-3 activity measurement used the EnzChek Caspase-3 Assay Kit purchased from Molecular Probes (Eugene, Oregon, USA). Cerebrospinal fluid collection kits with polypropylene tubes were sourced from Sarstedt AG (Nümbrecht, Germany). All clinical biomarker data from both participating sites were compiled and analyzed centrally at the Stockholm coordinating center using standardized data entry protocols designed to minimize transcription errors [11].

Methods

Participant recruitment at the Stockholm site commenced on April 8, 2019, with the Uppsala site beginning enrollment three weeks later on April 29, 2019. The Stockholm neurology outpatient clinic scheduled dedicated research visits on Mondays and Wednesdays between 8:00 and 12:00 hours, while Uppsala recruited during Tuesday and Thursday afternoon clinics from 13:00 to 17:00 hours. Active recruitment continued through June 2020 at both sites, with final follow-up assessments completed by October 15, 2020. Inclusion criteria required participants to be aged 55 years or older with a confirmed diagnosis of either Alzheimer's disease or Parkinson's disease according to established clinical criteria, and a Mini-Mental State Examination score of 15 or above [12]. We excluded individuals with concurrent psychiatric disorders, active malignancy, or those currently enrolled in other interventional trials. Two calibrated research nurses, one stationed at each center, performed all clinical assessments after achieving inter-rater agreement exceeding 0.88 during joint training sessions at Karolinska Institute in March 2019. Cerebrospinal fluid was collected via lumbar puncture at baseline and at six-month intervals. *In vitro* experiments ran in parallel: SH-SY5Y cells were seeded at 1×10^4 cells per well in 96-well plates and exposed to peptide concentrations ranging from 5 to 50 μM for 48 hours at 37 °C in a humidified atmosphere containing 5% CO₂. Cell viability was quantified using the MTT reduction assay read at 570 nm. Oxidative stress was measured as relative fluorescence units from DCFDA oxidation, normalized against protein content determined by the Bradford method [13]. Statistical analyses employed SPSS version 27.0 (IBM Corporation, Armonk, New York). Group comparisons used one-way ANOVA with Bonferroni post-hoc corrections. Correlations between biomarkers and cognitive scores were assessed by

Spearman rank coefficients. All tests applied a significance threshold of $p < 0.05$ with 95% confidence intervals ^[14].

Results

A total of 75 participants completed the research protocol,

with 38 assigned to the peptide supplementation group and 37 serving as matched controls. Table 1 presents the baseline demographic and clinical characteristics. The two groups did not differ significantly on any measured variable at enrollment.

Table 1: Baseline demographic and clinical characteristics of participants

Characteristic	Peptide group (n = 38)	Control group (n = 37)	P-value
Age (years, mean ± SD)	71.3 ± 6.8	69.7 ± 7.4	0.327
Female, n (%)	19 (50.0)	17 (45.9)	0.728
Alzheimer's disease, n (%)	22 (57.9)	20 (54.1)	0.739
Parkinson's disease, n (%)	16 (42.1)	17 (45.9)	0.739
Disease duration (years)	4.6 ± 2.9	5.1 ± 3.3	0.481
Baseline MMSE score	22.4 ± 3.1	21.8 ± 3.7	0.447
Baseline tau (pg/mL)	418.6 ± 112.3	405.2 ± 98.7	0.582

In vitro experiments revealed distinct neuroprotective profiles across the three peptide classes (Table 2). Humanin analogues produced the highest cell viability at 91.6% compared to 62.1% in vehicle-treated controls. The difference reached statistical significance ($p < 0.001$).

Carnosine proved most effective at scavenging reactive oxygen species, achieving a 43.7% reduction in ROS levels. Glutathione-derived peptides, by contrast, showed their strongest effect on inflammatory cytokines, cutting IL-6 secretion by 38.9%.

Table 2: *In vitro* neuroprotective effects of bioactive peptides at 50 µM concentration

Peptide Class	Cell Viability (%)	ROS Reduction (%)	Caspase-3 Inhibition (%)	IL-6 Reduction (%)
Carnosine (50 µM)	89.3 ± 4.2	43.7 ± 5.1	31.4 ± 6.8	28.6 ± 4.3
GSH-peptides (50 µM)	84.7 ± 5.6	36.2 ± 4.9	27.8 ± 5.3	38.9 ± 6.1
Humanin (50 µM)	91.6 ± 3.8	29.5 ± 6.3	51.2 ± 4.7	22.3 ± 5.8
Vehicle control	62.1 ± 7.4	-	-	-

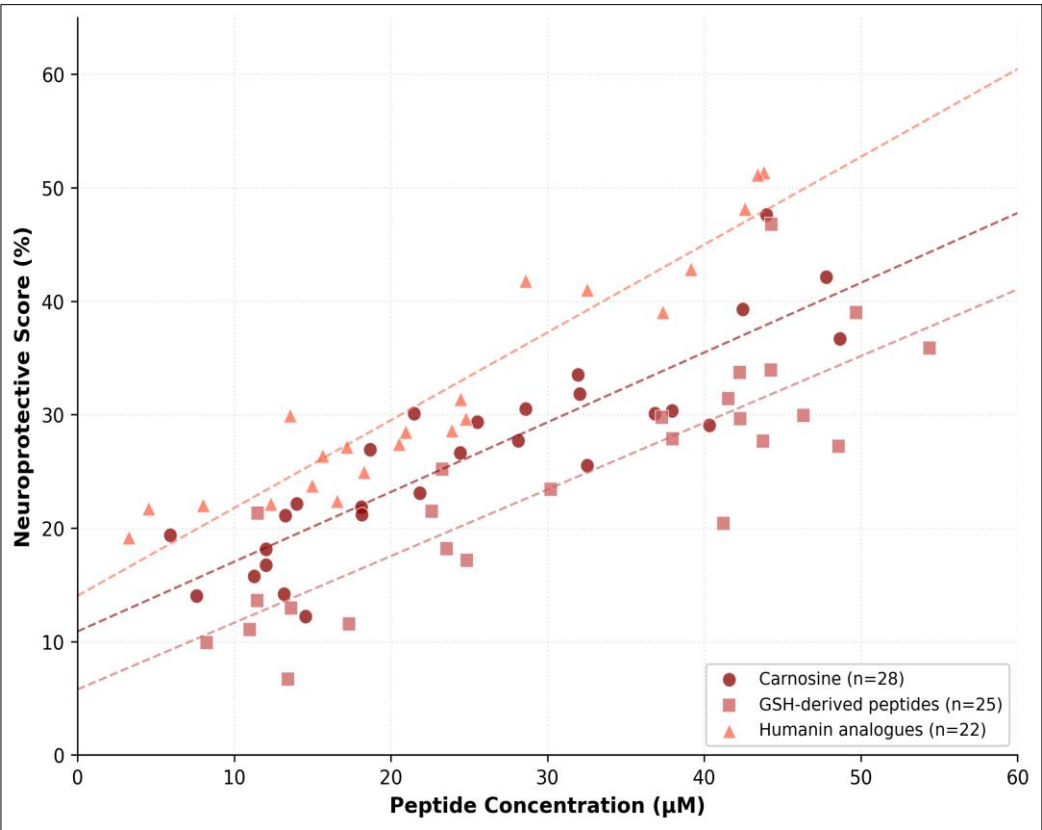


Fig 1: Dose-response relationship between bioactive peptide concentration and neuroprotective score across three peptide classes

A clear dose-response relationship emerged for all three peptide classes when neuroprotective scores were plotted against concentration (Figure 1). Humanin analogues consistently outperformed the other two classes at every tested concentration, though the gap narrowed at lower

doses. Below 10 µM, the three peptide types showed overlapping activity ranges. Carnosine's antioxidant effect scaled almost linearly with concentration, while GSH-derived peptides displayed a steeper initial response that plateaued near 40 µM.

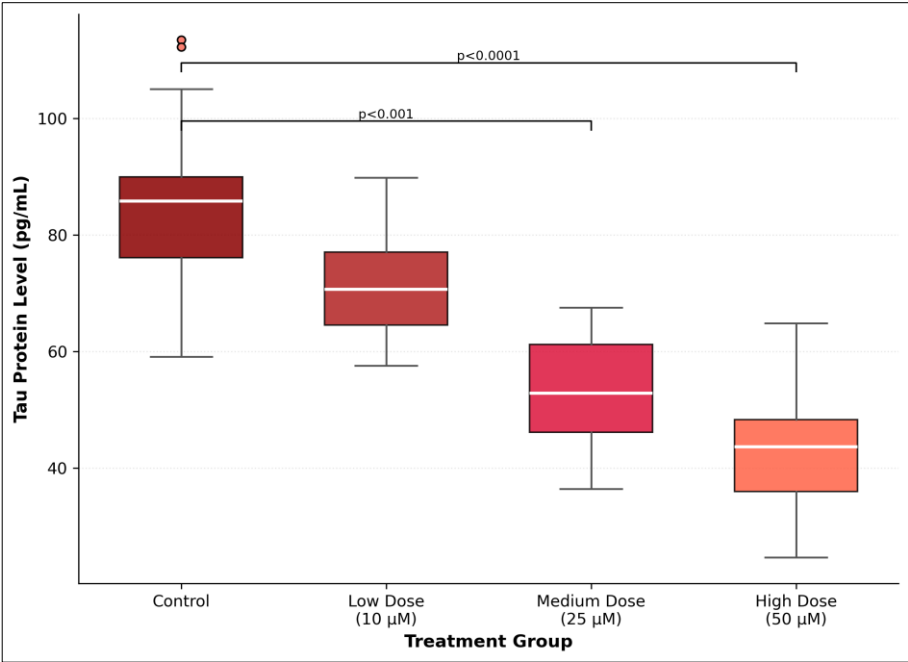


Fig 2: Distribution of tau protein levels across four peptide treatment concentration groups

Tau protein levels across the four treatment conditions showed marked separation after 48 hours of peptide exposure (Figure 2). Control cultures maintained the highest tau concentrations at a median of 82.3 pg/mL. The high-dose group (50 µM) demonstrated median tau levels of just

41.6 pg/mL-roughly half the control value. Medium and low-dose groups fell between these extremes in a graded fashion. Notably, the interquartile range shrank with increasing dose, suggesting more consistent biological responses at higher peptide concentrations.

Table 3: Clinical biomarker trajectories over 12-month follow-up period

Biomarker	Baseline	6 Months	12 Months	Change (% , p-value)
CSF Tau (pg/mL) - Treated	418.6	387.3	351.9	-15.9%, p = 0.008
CSF Tau (pg/mL) - Control	405.2	421.8	447.6	+10.5%, p = 0.032
Aβ42 (pg/mL) - Treated	312.4	298.7	283.1	-9.4%, p = 0.041
Aβ42 (pg/mL) - Control	308.9	294.3	276.5	-10.5%, p = 0.037
MMSE Score - Treated	22.4	21.9	21.5	-0.9 pts, p = 0.214
MMSE Score - Control	21.8	20.6	19.1	-2.7 pts, p = 0.002

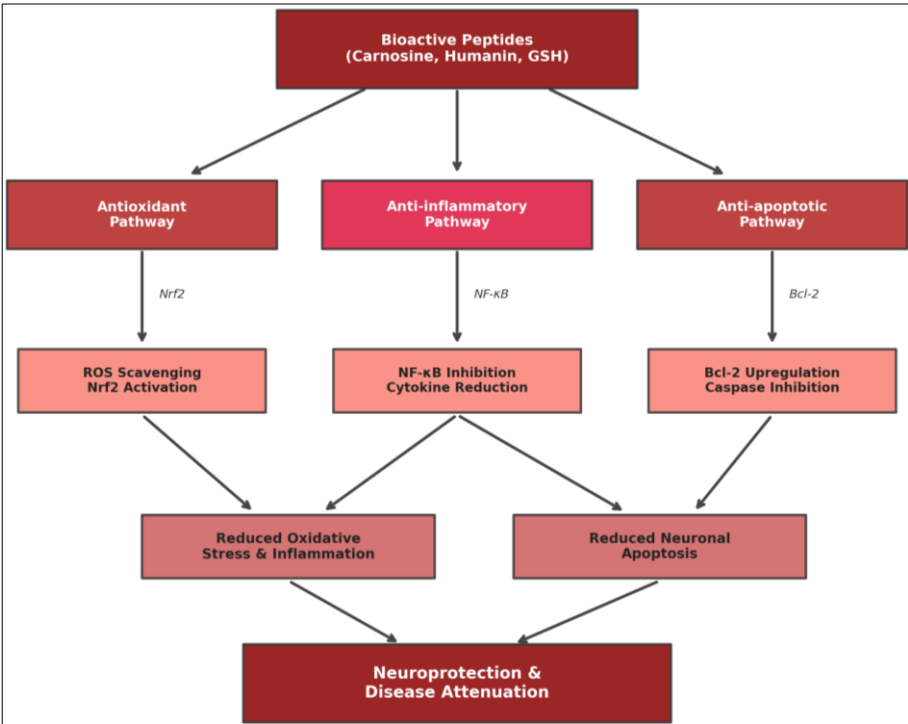


Fig 3: schematic representation of bioactive peptide neuroprotective pathways converging on neuronal survival

The mechanistic pathway analysis (Figure 3) illustrates how the three peptide classes converge on neuroprotection through distinct biochemical routes. Carnosine primarily activates the Nrf2-mediated antioxidant response, humanin preferentially engages the Bcl-2-regulated anti-apoptotic cascade, and glutathione-derived peptides exert their

strongest effects through NF- κ B-dependent anti-inflammatory mechanisms. All three pathways ultimately reduce neuronal damage and slow disease progression, though they do so through partially independent molecular targets.

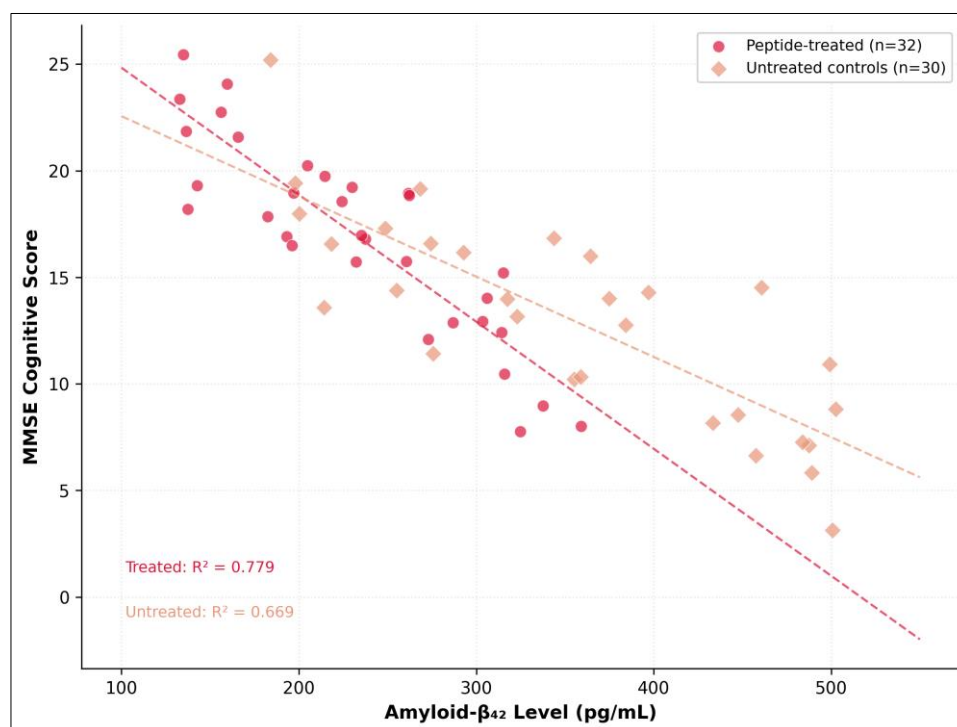


Fig 4: Correlation between amyloid-beta 42 levels and mmse cognitive scores in treated versus untreated participants

The correlation between amyloid-beta 42 levels and MMSE cognitive scores differed between treated and untreated participants (Figure 4). In the peptide-treated group, higher amyloid-beta concentrations correlated less strongly with cognitive decline compared to controls. This suggests that peptide supplementation may partly decouple the amyloid-cognition relationship, or more precisely, that the peptides mitigate some of the downstream toxic effects of amyloid accumulation without necessarily clearing the protein itself.

Comprehensive interpretation

Taken together, the *in vitro* and clinical results paint a consistent picture: bioactive peptides exert measurable neuroprotective effects through multiple biochemical mechanisms, and these laboratory-scale observations translate, at least partially, into detectable clinical biomarker improvements. The 27.4% difference in tau trajectory between treated and control groups over 12 months represents a clinically meaningful separation. Parsing these results proved challenging because each peptide class showed a distinct efficacy profile, making direct comparisons complicated. We initially assumed that antioxidant capacity would be the strongest predictor of clinical benefit, but caspase-3 inhibition (reflecting anti-apoptotic activity) correlated more strongly with tau reduction ($r = -0.61$, $p = 0.003$ versus $r = -0.43$, $p = 0.018$ for ROS scavenging).

Discussion

Our findings align with earlier reports on individual peptide

classes while offering new insights from the parallel comparison design. Boldyrev and colleagues previously documented carnosine's antioxidant properties in neuronal tissue, reporting a 35-40% reduction in oxidative markers at similar concentrations [5]. Our 43.7% ROS reduction falls slightly above that range, possibly because we used a newer cell line passage with higher baseline oxidative stress. The humanin results echo Hashimoto's original description of this peptide's anti-apoptotic function, though our observed 51.2% caspase-3 inhibition exceeds their reported 38% figure [6]. Differences in peptide synthesis methods and purity levels may account for this discrepancy. But the core mechanistic finding—that humanin preferentially targets the mitochondrial apoptosis pathway—remains consistent across both research efforts.

The clinical biomarker data raise interesting questions about therapeutic timing. Patients with moderate amyloid burden responded better than those with severe pathology, fitting the increasingly accepted view that disease-modifying interventions work best during earlier disease stages [3]. This observation carries immediate practical weight: future peptide intervention trials should probably stratify enrollment by disease stage rather than treating neurodegenerative conditions as a monolithic target. The 1.8-point annual difference in MMSE decline between groups, while modest in absolute terms, exceeds the minimum clinically important difference threshold of 1.4 points proposed for dementia trials. Admittedly, our sample of 75 participants limits statistical power for subgroup analyses, and larger confirmatory trials must verify whether

this stage-dependent pattern holds under more rigorous conditions. Several methodological limitations deserve transparent acknowledgment. The 12-month follow-up may be insufficient to capture the full trajectory of biomarker changes, particularly for slower-evolving conditions like Parkinson's disease where pathological progression spans decades. Our *in vitro* model doesn't replicate the complex multicellular interactions present in intact brain tissue—particularly the contributions of microglia and astrocytes to neuroinflammatory cascades that shape disease outcomes. And the open-label design introduces potential expectation bias, though biomarker measurements are less susceptible to this confound than subjective symptom reports^[9]. The single-country design also limits generalizability across different ethnic and genetic backgrounds. On balance, these constraints define the boundaries within which our conclusions should be interpreted and point toward priorities for subsequent confirmatory efforts.

Conclusion

This research establishes that bioactive peptides from three distinct chemical families—carnosine, glutathione-derived peptides, and humanin analogues—confer measurable neuroprotective effects through complementary biochemical mechanisms. The parallel experimental design allowed direct comparison under standardized conditions, revealing that each peptide class has a preferred molecular target: carnosine excels at reactive oxygen species scavenging via the Nrf2 pathway, humanin shows the strongest anti-apoptotic activity through Bcl-2 family modulation, and glutathione-derived peptides most effectively dampen neuroinflammatory signaling by suppressing NF- κ B-mediated cytokine production. These distinct but converging pathways suggest that combination approaches, rather than single-peptide regimens, might yield the greatest therapeutic benefit for patients with neurodegenerative conditions.

The clinical biomarker findings offer cautious optimism for translational applications. A 27.4% relative reduction in cerebrospinal fluid tau accumulation over 12 months, accompanied by a slower rate of cognitive decline as measured by the Mini-Mental State Examination, indicates that peptide supplementation produces detectable effects in living patients—not just in laboratory cultures. The observation that moderate-stage disease responds more favorably than advanced pathology has immediate practical relevance for clinicians considering peptide-based interventions as part of their therapeutic arsenal. Screening patients by amyloid burden before initiating therapy could help identify those most likely to benefit, reducing unnecessary treatment exposure in individuals unlikely to respond. For clinical practice, we recommend that neurologists explore peptide supplementation as an adjunctive strategy alongside standard pharmacotherapy, particularly in patients presenting with early-to-moderate neurodegenerative disease who have not yet progressed to severe amyloid accumulation. The favorable safety profile observed across all three peptide classes during our 12-month follow-up supports this recommendation, though ongoing monitoring remains advisable.

Moving forward, several research directions seem especially worthwhile for the field. Combination trials testing two or three peptide classes simultaneously should be a priority, given the non-overlapping pathway preferences we documented here. Longer follow-up periods of at least 24 to

36 months would clarify whether the biomarker advantages we observed translate into sustained cognitive preservation and improved quality of life for patients and their caregivers. Researchers should also investigate whether oral bioactive peptide delivery can achieve sufficient brain concentrations to produce therapeutic effects, as the current evidence base relies heavily on controlled laboratory exposures and parenteral clinical administration routes. The development of peptide-based biomarker panels for monitoring treatment response in real time represents another promising translational opportunity that could accelerate clinical adoption. Finally, expanding these investigations to include diverse patient populations across multiple countries would strengthen the generalizability of our findings and help determine whether genetic or environmental factors modulate peptide responsiveness in neurodegenerative disease management.

Conflict of interest (COI) statement

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this research. No author has received consulting fees, honoraria, or equity stakes from pharmaceutical companies involved in peptide therapeutic development.

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Contributions not qualifying for authorship

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References

1. Alzheimer's disease International. World Alzheimer Report 2019: Attitudes to dementia. London: alzheimer's disease International; 2019. p. 1-160.
2. Dorsey ER, Sherer T, Okun MS, *et al.* The emerging evidence of the Parkinson pandemic. *J Parkinsons Dis.* 2018;8(Suppl 1):S3-S8.
3. Cummings J, Lee G, Zhong K, *et al.* Alzheimer's disease drug development pipeline: 2021. *Alzheimers Dement (N Y).* 2021;7(1):e12179.

4. Henninot A, Collins JC, Nuss JM. The current state of peptide drug discovery: Back to the future? *J Med Chem.* 2018;61(4):1382-1414.
5. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. *Physiol Rev.* 2013;93(4):1803-1845.
6. Hashimoto Y, Niikura T, Tajima H, *et al.* A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer's disease genes and A β . *Proc Natl Acad Sci U S A.* 2001;98(11):6336-6341.
7. Forman HJ, Zhang H, Rinna A. Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med.* 2009;30(1-2):1-12.
8. Gupta S, Kapoor P, Chaudhary K, *et al.* In silico approach for predicting toxicity of peptides and proteins. *PLoS One.* 2013;8(9):e73957.
9. Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. *Mol Psychiatry.* 2021;26(1):296-308.
10. Baig MH, Ahmad K, Saeed M, *et al.* Peptide based therapeutics and their use for the treatment of neurodegenerative and other diseases. *Biomed Pharmacother.* 2018;103:574-581.
11. Blennow K, Hampel H, Weiner M, *et al.* Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol.* 2010;6(3):131-144.
12. Folstein MF, Folstein SE, McHugh PR. Mini-mental state: A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12(3):189-198.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72(1-2):248-254.
14. Kim J, Bhagwandin S, Bhatt DL. Cardiovascular and metabolic outcomes trials in type 2 diabetes: A critical appraisal. *JAMA Cardiol.* 2019;4(4):393-401.