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

Humanin and Humanin Analogs

Evidence Summary

Humanin is a mitochondrial derived peptide that has cytoprotective properties. It influences metabolism, promotes insulin sensitivity, and prolongs healthspan, but has unknown utility as a therapeutic in humans.

Neuroprotective Benefit: Humanin and analogs protect neurons by increasing their resilience to a variety of cell stressors and can inhibit toxic Aβ oligomerization.

Aging and related health concerns: Maintenance of humanin levels may prolong healthspan. Humanin promotes insulin sensitivity and metabolic adaptions following exercise, and may protect against ischemic cardiovascular damage.

Safety: Endogenous humanin is a safe peptide, but its effects can be context dependent, and may be sex dependent. Clinical human testing has not been conducted, and therapeutic dosing has not been established.

Availability: Research use	**Dose**: Not established	**Chemical formula:** $C_{119}H_{204}N_{34}O_{32}S_2$ **MW**: 2687.2 g/mol **Sequence**: H-Met-Ala-Pro-Arg-Gly-Phe-Ser-Cys-Leu-Leu-Leu-Leu-Thr-Ser-Glu-Ile-Asp-Leu-Pro-Val-Lys-Arg-Arg-Ala-OH
Half-life: 30 minutes (mice)	**BBB**: Varied/unclear	
Clinical trials: None	**Observational studies**: Humanin levels decline with age	

What is it?

Humanin (MAPRGFSCLLLLTSEIDLPVKRRA) is a **mitochondrial derived peptide** encoded by a small open reading frame within the 16S rRNA (MT-RNR2). It can be translated in the mitochondria or cytoplasm, and it is unclear which compartment translation takes place in humans [1]. When translated in the mitochondria, the peptide is 21 amino acids long, and 24 amino acids long when translated in the cytoplasm. There are also 13 humanin-like open reading frames found in the nuclear genome (MTRNR2L#), but their contribution is unclear [2]. While it lacks a classic signal peptide, humanin itself can act as a signal peptide for secretion. It is produced in response to cellular stress and has **cytoprotective activities** both intracellularly and extracellularly. It can influence a variety of signaling pathways including, JAK2, STAT3, MAPKs, and JNK. While it can engage several extracellular receptors, the cytoprotective effects are largely mediated through the humanin heterotrimeric receptor complex, which consists of ciliary neurotrophic factor receptor α (CNTFR), the cytokine receptor WSX-1, which acts as a receptor for IL-27, and the transmembrane glycoprotein gp130 (gp130), which is part of the IL-6 cytokine receptor complex. Humanin can influence cellular bioenergetics by altering mitochondrial activity. The critical residues in humanin responsible for its various activities have been mapped, and analogs have been produced to increase its potency and/or stability [3]. Although widespread benefits have been identified in preclinical studies, humanin has not yet undergone clinical translation.

Major humanin analogs:

S14G-HN (**HNG**) (MAPRGFSCLLLLT**G**EIDLPVKRRA) is a humanin analog where the serine at position 14 is substituted for a glycine [3]. The serine at 14 is one of the critical residues for neuroprotective activity, which is abolished when it is converted to an alanine, but enhanced when converted to a glycine. This substitution makes HNG 1000 times more potent than humanin in activating the humanin heterotrimeric receptor. HNG is the major humanin analog used in preclinical research studies.

HNGF6A (MAPRG**A**SCLLLLT**G**EIDLPVKRRA) is a humanin analog which contains the S14G substitution to increase potency as well as a substitution of an alanine in place of the phenylalanine at position 6 [3]. This phenylalanine is critical for the binding of humanin to IGFBP-3 [4]. The interaction between humanin and IGFBP-3 influences the clearance rate, thus HNGF6A has superior stability and pharmacokinetics relative to IGFBP-3 binding analogs [5]. HNGF6A is more potent at modulating insulin action.

Colivelin (SALLRSIPA- PAGASRLLLLTGEIDLP) is the most potent humanin derivative developed to date. It shows neuroprotective activity at the fM range and is 10^3 to 10^7 more potent than humanin and other humanin analogs [6]. It is a combination of the potent humanin analog AGA-(C8R)-HNG17 with the C-terminus of activity dependent neurotrophic factor ADNF (SALLRSIPA). AGA-(C8R)-HNG17 (P**A**G**A**S**R** LLLLT**G**EIDLP) was identified through mutational analysis. Relative to humanin, it is missing the first 3 amino acids and the last 5 amino acids, and contains substitutions at amino acids 4 (R4A), 6 (F6A), 8 (C8R), and 14 (S14G), which confers it with increased potency and stability. ADNF also has neuroprotective activity, and the fusion of the humanin analog with ADNF results in synergistic neuroprotection. Structurally, colivelin is more similar to ADNF than humanin [7].

Neuroprotective Benefit: Humanin and analogs protect neurons by increasing their resilience to a variety of cell stressors and can inhibit toxic Aβ oligomerization.

Types of evidence:

- 1 biomarker study (humanin CSF levels decreased in AD)
- 1 gene association study (Humanin SNP associated with cognitive aging)
- Numerous laboratory studies

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

Age-related cognitive decline: SNP THAT DECREASESE HUMANIN ASSOCIATED WITH FASTER COGNITIVE AGING

A mitochondrial gene association study identified a single nucleotide polymorphism (SNP) (MitoG2706A/rs2854128) within the humanin coding region that is associated with accelerated cognitive aging, though the effect is impacted by race [8]. The SNP is less common in Caucasian Americans relative to African Americans, as the ancestral/reference allele was present in 42% of

Caucasian Americans, but only 1.7% of African Americans, and 6% of Hispanics, in this study. This SNP is associated with a 14% decrease in circulating levels of humanin, and may account for the decreased average level of circulating humanin in African Americans (1211.9 ± 222.7 pg/mL) relative to Caucasian Americans (1491.0 ± 267.3 pg/mL). The impact of the SNP on cognitive aging was found to be affected by race, as the presence of this SNP accelerated aging by 2 years in the African American cohort, but only by 0.2 years in the Caucasian American cohort.

Human research to suggest benefits to patients with dementia:

Alzheimer's disease: HUMANIN DECREASED IN AFFECTED AD BRAIN REGIONS

Humanin was originally discovered from a screen to identify differentially expressed factors between preserved and affected brain tissue in the Alzheimer's disease (AD) brain [9]. It was found to be expressed in the relatively spared occipital lobe, and was hypothesized to be a factor that can preserve brain function in people with AD. Circulating CNS levels of humanin are reduced in AD patients, relative to the cerebrospinal fluid (CSF) levels in age-matched controls [10]. Although it has been proposed as a therapeutic for AD since its discovery, it has not yet been tested as a therapeutic agent in humans.

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

Alzheimer's disease: POTENTIAL BENEFIT (Preclinical models)

Humanin and humanin analogs have been shown to be protective against mitochondrial dysfunction, and neuronal loss in a variety of preclinical AD models.

Humanin (100 umol/L intrahippocampal) increased dendritic complexity, synaptic protein levels, and synaptic plasticity in Aβ42 injected rats [11]. Humanin treatment also reduced oxidative stress, neuronal apoptosis, and tau hyperphosphorylation.

HNG treatment (0.1 ug i.p. every other day for 3 months) starting at 9 months of age, when APP/PS1 mice already have plaques and neurological deficits, reduced Aβ plaque deposition, glial activation, pro-inflammatory mediators (IL-1, IL-6, TNFα), and improved spatial memory, based on performance on the Morris water maze [12]. HNG (50 or 100 ug/kg i.p.) also improved brain insulin sensitivity, and enhanced autophagic flux in symptomatic APP/PS1 mice [13]. In the diazepam-induced memory deficit mouse model, HNG pretreatment (200 nM i.p.) prevented memory impairments, but did not impact anxiety phenotypes [14].

Colivelin protected against learning and memory impairments in a variety of AD models. Colivelin (0.2 nmol intrahippocampal) protected against Aβ25-35 induced learning and memory deficits in rats [15]. Intranasal colivelin (1 nmol/day for 3 weeks) improved performance on learning and memory tasks (the novel object recognition task, Morris water maze) in symptomatic 9-month-old APP/PS1 mice [16]. Colivelin (20 ug/day i.p. for 6 days) reduced the deposition of Aβ, neuroinflammation, neuronal cell loss, and spatial memory impairments in 19-month-old PDAPPV717I AD model mice [17]. The therapeutic effect was partially due to the inhibition of p38 MAPK. Intranasally administered colivelin reduced Aβ-induced memory impairments through the activation of STAT3 signaling, and STAT3-mediated promotion of cholinergic neurotransmission. Intranasal colivelin (5 nmol every other day for 3 weeks) also restored cognitive function in the Tg2576 and intracerebroventricular Aβ42 mouse models of AD through activation/restoration of the JAK2/STAT3 signaling and restoration of cholinergic neurotransmission [18].

Cytoprotection: HUMANIN PROMOTES NEURONAL SURVIVAL

Humanin can enhance cell viability through both intracellular and extracellular mechanisms. Humanin can act intracellularly to inhibit apoptotic cell death by binding to the pro-apoptotic mediators Bax and cBid in the cytosol, which prevents their translocation to mitochondrial membranes, which is necessary for their ability to trigger mitochondrial-dependent apoptosis [19]. Secreted, extracellular humanin can also induce cytoprotective signaling pathways in an autocrine or paracrine manner by binding to extracellular receptors. Humanin can bind to FPRL1 or FPRL2 to activate pro-survival ERK signaling, and induce calcium mobilization. Humanin also binds to the heterotrimeric receptor complex, gp130, CNTRF, and WSX-1, leading to activation of JAK2/STAT3, Akt/P13K, ERK1/2, as well as the inhibition of JNK, and modulation of various other MAPKs, in a cell type/context dependent manner [2; 20]. These converge on a cell survival phenotype. Humanin also promotes cell survival through its interactions with IGFBP-3, a modulator of cell growth and survival. Humanin can inhibit IGFBP-3 mediated cell death, but the effect can be cell type dependent [21]. Thus, **humanin increases the resistance of cells toward a variety of cellular stressors**, including Aβ.

Amyloid toxicity: HUMANIN INHIBITS AMYLOID AGGREGATION

Humanin is associated with the reduction of Aβ fibrils, but it does not affect Aβ production or APP processing [22]. Instead, humanin and humanin analogs can act as chaperones to limit the growth of amyloid oligomers and prevent the formation of large aggregates by binding to misfolded, seeding competent amyloid oligomers [23; 24; 25].

Mitochondrial function: HUMANIN ENHANCES BIOENERGETICS

Mitochondrial derived peptides help maintain mitochondrial homeostasis and modulate cellular metabolism. Humanin influences mitochondrial bioenergetics by increasing the basal oxygen consumption rate, which allows a cell to maintain ATP production and mitochondrial reserve capacity in the context of cellular/oxidative stress [26]. Correspondingly, it can prevent the loss of mitochondrial membrane potential in response to oxidative stress.

Autophagy: HUMANIN ENHANCES TRANSFER OF SUBTRATES TO LYSOSOME (Preclinical)

Humanin can activate chaperone mediated autophagy by increasing the translocation of substrates into lysosomes, but it does not affect overall autophagic flux [27]. Humanin interacts with the chaperone protein HSP90 at the lysosomal membrane and stabilizes its binding of substrates. This activity is associated with the increased survival of neurons and other cell types in response to cellular stressors in cell culture.

Amyotrophic lateral sclerosis: POTENTIAL BENEFIT (Preclinical model)

Colivelin treatment (10 pmol or 1 nmol per 2 days i.c.v.) starting at 80 days old, prolonged motor neuron survival and motor performance at 120 days in the $SOD1^{G93A}$ mouse model [28]. Colivelin (1 nmol) delayed the onset of disease by about two weeks, and prolonged survival accordingly.

Menopause-associated cognitive dysfunction: OVARIAN HORMONES PROMOTE HUMANIN RELEASE (Preclinical)

Ovariectomized female rats exhibit decreased expression of humanin in the hippocampus, as well as decreased performance on cognitive tests [29]. Humanin expression was primarily localized to astrocytes, and the decrease in humanin was associated with reduced astrocyte complexity in the ovariectomized rats. In cell culture, ovarian hormones increase the expression and release of humanin by astrocytes. Humanin preserved synaptic architecture and function in hippocampal neurons, suggesting that the loss of ovarian hormone induced humanin contributes to hippocampal atrophy and cognitive decline. Humanin supplementation may then be protective against menopause-associated cognitive dysfunction.

Age-related cognitive dysfunction: POTENTIAL BENEFIT (Preclinical models)

HNG treatment (4 mg/kg i.p. biweekly for 6 to 10 months) starting at middle age (8-month-old) in female mice slowed age-related cognitive decline [8]. When assessed at 28 months old (elderly), the

treated mice showed better performance on the Barnes maze and Y-maze cognitive tests, relative to their untreated counterparts. HNG treated mice also had better balance and coordination as they aged, suggesting it has broad effects across the nervous system.

APOE4 interactions: Unknown

Aging and related health concerns: Maintenance of humanin levels may prolong healthspan. Humanin promotes insulin sensitivity and metabolic adaptions following exercise, and may protect against ischemic cardiovascular damage.

Types of evidence:

- 2 exercise intervention clinical trials (Effects on humanin levels)
- 9 biomarker studies (Humanin levels in aging or disease)
- Numerous laboratory studies

Lifespan and healthspan: HUMANIN EXTENDS HEALTHSPAN & POTENTIALLY LIFESPAN

Healthspan: The maintenance of humanin levels is associated with the prolongation of healthspan, however, it is unclear whether humanin alone can meaningfully impact lifespan in humans. In most of the species assessed, **circulating humanin levels decrease during old age**. Humanin levels decrease over 2-fold in rhesus macaques between 19 and 25 years of age, by approximately 40% by 18 months of age in mice, and by approximately one-third in humans over age 80 [10; 30]. Levels of the rat homolog, rattin, also decrease with age [30]. The period when humanin levels fall most dramatically corresponds with the period that each respective species typically experiences aging-related conditions/a decline in healthspan. Notably, the naked mole rat, which is known for a minimal senescence phenotype and exceptional healthspan, experiences only a marginal decline in humanin levels during aging, suggesting that **the maintenance of humanin may contribute to their prolonged healthspan** [10]. Since humanin is typically elevated in response to acute stressors, humanin levels would be expected to increase under conditions of elevated stress, such as disease or aging. Some studies have found elevated levels in certain disease populations [31; 32; 33] and aging cohorts [34] suggesting that there may be a transition period whereby humanin is initially elevated as a protective response, but then under chronic conditions maladaptation results in a downregulation or unproductive alteration of the humanin response.

Lifespan: Transgenic overexpression of humanin in *C. elegans* under the ife-1 promoter increased lifespan from 17.7 to 19 days ($p<0.05$), which was dependent on daf-16/FOXO [10]. These worms had reduced body size, body fat, and reproductive output. Transgenic overexpression of humanin in mice under the CMV promoter, also decreased body size, body fat, and reproductive output. These mice had 16% higher levels of circulating humanin, and were protected against chemotherapy-associated cellular toxicity. Treatment with the humanin analog HNG (4 mg/kg i.p. biweekly) starting at 18 months of age did not increase the lifespan of the animals, but it did improve healthspan. Endogenous circulating levels are associated with lifespan, as **humanin levels have been found to be elevated in centenarians** [34] and to be approximately two-fold higher in the children of centenarians, relative to age-matched controls [10]. These studies suggest that humanin-mediated lifespan extension may require elevated humanin levels throughout the duration of the lifespan. However, humanin treatment, even late in life still has the potential to improve healthspan [10].

The effects on lifespan and healthspan are related to **humanin's effects on IGF-1 signaling** [10]. Humanin levels are inversely correlated with growth hormone and IGF-1. Humanin levels and lifespan are decreased in mice that overexpress growth hormone, while humanin is increased 40% and lifespan is extended in Ames dwarf mice, which have marginal expression of growth hormone and IGF-1 [35]. IGF-1 acts as a negative regulator of humanin. Humanin can also reduce circulating levels of IGF-1 by binding to IGFBP-3, a carrier protein that affects the stability and bioavailability of IGF-1 [36]. In mice, treatment with IGF-1 reduces humanin levels by 30% [35]. Children deficient in growth hormone have elevated humanin levels, which are inversely correlated with IGF-1 levels (Pearson's correlation coefficient of -0.69, $P < 0.05$), and the initiation of growth hormone treatment led to a 20% reduction in humanin levels. Similarly, a cohort from Ecuador with growth hormone receptor deficiency, with extremely low levels of IGF-1, had humanin levels 80% higher than their non-affected relatives. Although there was no clear association with lifespan, individuals from the Ecuadorian cohort with low IGF-1 were more likely to die from accidents than age-related diseases, compared to their relatives [37]. An inverse correlation of humanin levels and IGF-1 was also found in a healthy aging cohort (Spearman rank correlation: $\rho = -0.259$; $p = 0.002$) [34].

Senescence: POTENTIAL MIXED (Cell culture models)

The role of humanin and other mitochondrial derived peptides in the onset and maintenance of cellular senescence is unclear, as some studies report features associated with the inhibition of senescence [38], while others indicate that these peptides can drive the pro-inflammatory senescence associated phenotype (SASP) [39]. This suggests that the effects of mitochondrial derived peptides on senescence

phenotypes are complex, and likely to be both cell type and context dependent, due to differences in receptor coupling and thus downstream signaling.

Cancer: POTENTIAL MIXED (Preclinical models)

There are conflicting reports regarding humanin's role in tumor progression and chemoresistance [40]. Humanin is upregulated in gastric cancer and bladder cancer, with the opposite anticipated effects in promoting and inhibiting tumor progression, respectively. In some preclinical models, HNG has been shown to protect healthy cells from chemotherapeutic toxicity, without impeding anti-tumor efficacy, suggesting it may be beneficial as an adjunct to chemotherapy. However, other preclinical studies show that systemic humanin treatment promotes tumor progression. These findings suggest that the effects of humanin are cancer type dependent, related to the signaling pathways that promote tumor growth in various cancers. Humanin activates cell survival pathways known to be involved in cancer, including ERK1/2, AKT, and JAK2/STAT3. However, in the Ecuadorian growth hormone deficiency cohort, which have been shown to have elevated humanin levels, the incidence of cancer was significantly lower than the average population [37]. Overall, evidence regarding the oncogenic potential of endogenous humanin is scarce, but caution is warranted for exogenous therapeutic use until more information is available.

Age-related macular degeneration: HUMANIN ENHANCES CELL SURVIVAL (Cell culture)

In a cell culture model, ARPE-19 transmitochondrial cybrid cells, mitochondria from patients with age-related macular degeneration or age-matched healthy controls were inserted into the ARPE-19 cells [41]. The cybrid cells containing mitochondria from patients with macular degeneration had reduced cell viability, lower copies of mtDNA, higher levels of mitochondrial fragmentation, and reduced antioxidant capacity. Treatment of the cybrid cells with HNG restored cell viability, by reducing mitochondria-mediated apoptosis. The effect was related to a reduction in pro-apoptotic proteins (Bax), and activation of the JAK2 pro-survival pathway.

Hair loss: HUMANIN PROMOTES HAIR GROWTH (Preclinical models)

The humanin analog, HNG, has been shown to promote the growth phase of hair follicles through the activation of ERK1/2, AKT, STAT3 signaling pathways, leading to the upregulation of the growth phase maintenance factor, VEGF [42]. In rat vibrissa hair follicle organ culture, HNG treatment increased hair shaft elongation by 164%. HNG (100 nmol) applied to the shaved back of mice, significantly increased hair growth within 35 days.

Osteoporosis: HUMANIN PROMOTES BONE FORMATION (Cell culture model)

In mouse osteoblastic cell culture (MC3TC-E1 cells), the humanin analog HNGF6A, has been shown to promote the differentiation of bone forming osteoblasts [43]. The osteogenic effects were related to the inhibition of p38 and JNK signaling pathways.

Cardiovascular disease: POTENTIAL BENEFIT (Preclinical models)

Myocardial ischemic injury: HUMANIN LIMITS DAMAGE/PRESERVES FUNCTION

Humanin and humanin analogs have been shown to be protective against myocardial ischemic injury in preclinical models by preventing oxidative stress-related cell death. In female minipigs, HNG (2 mg/kg 10 minutes prior to reperfusion) reduced myocardial infarct size by 41% following ischemic/reperfusion injury [44]. The cardioprotection was attributed to decreased cell death, as HNG had no effect on injury or oxidative stress markers. In male rats, HNG (252 µg/kg i.v.) administered during the ischemic period, decreased cardiac arrhythmia, infarct size, mitochondrial dysfunction, and left ventricular dysfunction [45]. The effects were related to the activation of cell survival pathways, including AKT, and a reduction in mitochondrial-mediated apoptosis. Humanin is highly expressed in the heart, and HNG treatment also led to an increase in levels of endogenous humanin within the myocardium. In mice, intracardiac administration of HNG (2 mg/kg) at the time of reperfusion reduced myocardial infarct size by 47%, and improved cardiac function based on left ventricle ejection fraction [46]. Once again, the protective effects were attributed to improved cardiomyocyte survival.

These studies suggest that humanin analogs may reduce the extent of damage following myocardial ischemic injury when administered to the heart during the ischemic period.

Stroke: HUMANIN LIMITS DAMAGE/PRESERVES FUNCTION

Similar to myocardial ischemic injury, humanin analogs preserve brain tissue and neurological function following models of cerebral ischemic injury. In male mice, HNG pretreatment administered directly to the CNS (0.1 µg i.c.v. 30 minutes prior) or peripherally (1 ug i.p. 1-hour prior), reduced cerebral infarct volume by 54% and 36%, respectively [47]. HNG post-treatment (0.1 ug i.c.v.) after four hours of reperfusion was less effective, as it reduced infarct volume by 19%. Colivelin pre-treatment (1 mg/kg i.p. 30 minutes prior) also reduced infarct size [48]. Pre-treatment with HNG or colivelin reduced neurological deficits in the mouse middle cerebral artery occlusion (MCAO) model. Based on rodent models, the neuroprotective effect stems from the enhancement of the STAT3 cell survival pathway, and a reduction in apoptotic signaling (Bax, caspase-3) [49]. HNG (2.5 µg i.p. 1-hour post injury) also

reduced neurological deficits and brain edema in a mouse model of intracerebral hemorrhage [50]. In this model, protection was mediated by activation of AKT/PI3K signaling.

Atherosclerosis: HUMANIN PRESERVED ENDOTHELIAL FUNCTION

Vascular endothelial inflammation is a driving factor in atherosclerosis. Individuals (n=40) with reduced coronary blood flow (change in CBF = −33 ± 25%), indicative of coronary endothelial dysfunction, had lower serum humanin levels (1.3 ± 1.1 vs. 2.2 ± 1.5 ng/mL, P = 0.03) relative to those with intact coronary endothelial function (change in CBF 194 ± 157%) [51]. Since humanin levels are typically responsive to conditions of cell stress, it suggests that similar to what is seen in individuals with other metabolic diseases, humanin expression and secretion may be altered in individuals with atherosclerotic progression. Under these conditions, there appears to be a disconnect between tissue and circulating humanin levels. Within the vascular tissue itself (n=34), humanin levels are higher in those with unstable atherosclerotic plaques (29.42±2.05 vs. 14.14±2.13% of plaque area, p<0.0001) relative to those with asymptomatic stable plaques [52]. The higher humanin levels may be due in part to increased vascular macrophage infiltration, as macrophages have been shown to express and secrete humanin.

Although the contribution of mitochondrial vs nuclear derived humanin isoforms in various tissues remains unclear, there may also be a change in the expression or distribution of humanin isoforms. In an epigenome wide association study, differential methylation in the promoter region of the nuclear encoded humanin isoform, MTRNR2L8 (HN8), was associated with large artery atherosclerotic stroke in a Chinese Han population (mean methylation difference −13.01%, $P=8.86\times10^{-14}$) [53].

Preclinical models similarly suggest that humanin and its analogs can preserve endothelial function and inhibit the progression of atherosclerosis. In ApoE deficient mice fed a high cholesterol diet, HNGF6A treatment (0.4 mg/kg/day i.p. for 16 weeks) preserved the expression of endothelial nitric oxide by decreasing reactive nitrogen species and mitigating oxidative stress [54]. In cultured human aortic endothelial cells, humanin treatment suppressed free fatty acid-induced oxidative stress by inhibiting the production of reactive oxygen species and protein carbonyls, as well as promoting the activation of AMPK [55]. When administered to old mice (18 months old), the humanin analog, HNG (4 mg/kg i.p. 2 times/week for 14 months), reduced myocardial fibrosis, including collagen deposition, MMP-2 expression, and fibroblast proliferation [56]. The anti-fibrotic effect may be mediated by the activation of AKT and GSK-3β.

Diabetes: HUMANIN LEVELS REDUCED IN TYPE 2 DIABETES; ENHANCES INSULIN SENSITIVITY

Humanin is protective for diabetes as **it improves insulin sensitivity**, and promotes the survival of pancreatic beta cells. However, metabolic syndromes are associated with a disruption in the endogenous activation of these protective pathways. Acute metabolic stress promotes an increase in humanin, however, chronic metabolic stress may lead to maladaptions that interfere with this process [57]. A small study (n=62) found that humanin levels were higher in men with type 1 diabetes, which may be a protective response due to elevated pancreatic stress [32]. The effect was not significant in women, which may stem from the influence of ovarian hormones on humanin levels. Meanwhile, serum humanin levels were found to be decreased in a study including type 2 diabetics (n=225), and the levels were inversely correlated with glycated hemoglobin (HbA1c) ($P < 0.0001$), which is an indicator of high blood glucose, and poorly managed diabetes [58]. Humanin levels were already reduced in individuals with pre-diabetes. Individuals (n=81) with impaired fasting glucose had reduced humanin levels compared with controls (124.3 ± 83.91 pg/mL vs 204.84 ± 92.87 pg/mL) [59]. The difference between humanin levels in type 1 and type 2 diabetes, may stem from the different origins of the diseases, as type 2 typically results from metabolic syndrome.

Preclinical models suggest that humanin may be beneficial for both type 1 and type 2 diabetes. Humanin regulates insulin by activating hypothalamic STAT3 signaling [30]. The peripheral effects of humanin on glucose uptake and insulin sensitivity can be achieved via intracerebroventricular administration of the humanin analog F6AHN, suggesting that the effect of humanin on insulin is centrally mediated [30]. HNGF6A increased glucose-stimulated insulin secretion in isolated islets 3-fold in control mice and 2.5-fold in diabetic mice by increasing the glucose oxidation rate [60]. Hypothalamic levels of the humanin homolog in rats, rattin, have been shown to decrease with age, suggesting that declines in CNS humanin may impact age-related declines in glucose homeostasis [30]. In the type 2 diabetes model, Zucker diabetic fatty rats, HNGF6A reduced (normalized) glucose levels by approximately 50% [30]. In the type 1 diabetes model, nonobese diabetic (NOD) mice, humanin treatment normalized glucose tolerance, reduced the infiltration of lymphocytes to the pancreas, and delayed the onset of disease [61].

Metabolic syndrome/obesity: HUMANIN REDUCES METABOLIC DYSFUNCTION

Mitochondrial derived peptides, such as humanin, are metabolic signal transducers, and act a part of the signaling network that transmits information about mitochondrial status to the rest of the cell [26]. These peptides serve to promote adaptive responses to metabolic stress as part of mitohormesis, the process by which low levels of ROS lead to positive adaptive mechanisms in mitochondria. However, in

the context of chronic metabolic stress, such as overnutrition, some of these mitochondrial adaptations may become maladaptive.

Humanin signaling influences insulin sensitivity as well as lipid utilization, which **affects the overall metabolic state**. The effects on metabolic parameters are not associated with a change in food consumption. In a model of diet-induced obesity, the plasma metabolite profile in male mice was altered in response to treatment with HNG (2.5 mg/kg 2x/day for 3 days) [62]. There was a significant change in 52 metabolites, with 14 increased and 38 decreased. The top differential metabolites between untreated and treated mice were 1-(3-aminopropyl)2-pyrrolidone (CID 82111), 2-aminobutyrate (CID 439691), and N-formylmethionine (CID 911). The major metabolic pathways affected were the methionine cycle and glutathione metabolism, gamma-glutamyl-amino acid, sphingolipid metabolism, and acylcarnitine metabolism. The effects on glutathione and sphingolipids are of particular interest because glutathione is an endogenous antioxidant, and some bioactive sphingolipids, such as sphingomyelin and S1P, are elevated in obese individuals with cardiovascular disease and poor glycemic control.

Preclinical studies indicate that humanin analog treatment can **ameliorate metabolic dysfunction in animal models**. In male mice with high-fat diet induced fatty liver, HNG (2 mg/kg i.p. for 4 weeks) reduced visceral fat and hepatic triglyceride accumulation by increasing the activity of hepatic microsomal triglyceride transfer protein and thus hepatic triglyceride secretion [63]. Similar to the central effect on insulin, the effect on hepatic triglyceride secretion appears to be a neurologically mediated effect driven by the central melanocortin system.

Humanin may protect against age-related metabolic changes, as mice treated with HNG (4 mg/kg i.p. biweekly) starting in old age (18 months) had reduced visceral fat and increased lean body mass for at least the next 10 months (duration) of their lives [10].

Exercise mimetic: HUMANIN INCREASES IN RESPONSE TO ACUTE EXERCISE

Humanin levels increase in response to acute exercise, which may promote the metabolic adaptations associated with exercise, such as improved mitochondrial efficiency. The exercise-associated effect on humanin primarily takes place within the skeletal muscle. Humanin levels in the plasma and skeletal muscle increase following an acute bout of high intensity exercise [64]. The increased protein levels are related to increased protein stability/decreased degradation, as there was no effect on mRNA expression levels**. Humanin levels are elevated in response to muscle contraction**, as the contraction of isolated mouse muscle led to an approximately 4-fold increase in humanin [64]. This relationship

between muscle contraction and humanin may explain why **humanin levels are most responsive to resistance training.** In prediabetic men (n=55, aged 40-65), 12 weeks of resistance training led to a 35% increase in skeletal muscle humanin levels [65]. There was also a weak correlation between serum humanin levels and glucose tolerance in the resistance trained group. Although acute bouts of various exercises can temporarily raise humanin levels, only resistance training appears to lead to sustained changes. Nordic walking and high intensity interval training (HIIT) for 12 and 6 weeks, respectively, did not significantly increase humanin levels [64; 65]. The increase in humanin with resistance training was associated with a 1.8-fold ± 0.47 increase in citrate synthase activity, the first enzyme of the TCA cycle, which drives oxidative phosphorylation, suggesting an increase in mitochondrial bioenergetics, and energy (ATP) production [65].

Safety: Endogenous humanin is a safe peptide, but its effects can be context dependent, and may be sex dependent. Clinical human testing has not been conducted, and therapeutic dosing has not been established.

Types of evidence:

- Several laboratory studies

The endogenous peptide humanin is generally considered to have beneficial effects in the body. It produces mitochondrial adaptations in the context of acute stress to preserve metabolic function and cell viability. However, the effects of humanin are cell type dependent, because humanin can engage several extracellular receptors, and the downstream effects depends on the signaling pathways coupled to the receptors as well as the presence of other signaling activators in the cellular environment [20]. Humanin levels and responses become disrupted in some chronic conditions, and it is hypothesized that under some contexts that elevated humanin levels become maladaptive. Due to its antagonistic relationship with growth hormone/IGF-1, sustained overexpression of humanin during developmental periods impedes growth and reproductive capacity [10; 35]. Exogenous supplementation of humanin and humanin analogs during adulthood has been shown to exert advantageous metabolic adaptations in animal models [57], but these studies have not adequately addressed the safety of long-term use. Cell culture and rodent studies suggest that under some conditions, humanin may promote tumor progression, and senescent/inflammatory phenotypes [40]. Further studies are needed to determine the conditions under which humanin supplementation could have negative effects.

Sex effect: Several studies have indicated that humanin may be differentially regulated in males and females, which may be related to the ability of ovarian hormones to induce humanin [29; 32]. This suggests that humanin may work differently or require a different dosing regimen when used in premenopausal women relative to men or post-menopausal women. Since the vast majority of preclinical studies testing humanin supplementation as a therapeutic have used male animals, it is unclear how well these findings translate to females, and more research is needed to address this issue.

Pharmacokinetics: One of the roadblocks to developing humanin as a therapeutic may be its pharmacokinetic (PK) prolife [40]. The kinetics and half-life of humanin analogs is dependent on their IGFBP-3 binding capacity. Circulating IGFBP-3 influences the peak level, duration, and clearance rate of exogenous humanin and humanin analogs [5]. The half-life of HNG in wildtype mice is only 30 minutes, but the PK profile appears to be species dependent, as exogenous humanin analogs had a half-life of >4 hours in rats. Based on the beneficial effects seen in animal models with once daily dosing, it suggests half-life in tissues may be significantly longer than the plasma half-life. However, a biodistribution study of exogenous humanin (via i.p.) in rats found that it was primarily found in the plasma, and to a much lesser degree in the liver, with none detectable in the brain or heart. It is unclear how the biodistribution of exogenous humanin is affected by the route of administration.

Humanin supplementation has not been tested in any well-controlled clinical trials. There were no specific reports of adverse reactions to humanin, although injection site reactions are possible.

Drug interactions: Interactions have not been established, but based on its known activities, humanin and its analogs may interact with the efficacy of growth hormone treatment, or cancer drugs targeting ERK signaling. Due to its insulin sensitizing effects, it may interact with drugs that impact glycemic control (i.e., antidiabetic agents).

Sources and dosing:

Humanin and humanin analogs are available for research use from commercial suppliers. They have not been developed or approved for human use.

Research underway:

Humanin and humanin analogs continue to be researched in preclinical studies, but there are no clinical studies underway. Humanin is being used in clinical trials as a biomarker.

Search terms:

Pubmed, Google: Humanin

- Alzheimer's disease, neurodegeneration, aging, lifespan, cardiovascular, diabetes, metabolism, exercise, cancer, pharmacokinetics, safety

Websites visited for Humanin:

- Clinicaltrials.gov
- PubChem

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