Older more fit KL-VS heterozygotes have more favorable AD-relevant biomarker profiles

Mackenzie Jarchow, BS[1], Ira Driscoll, PhD[1,2,*], Brianne M. Breidenbach, PhD[1,2], Noah Cook, MS[1,3,]
Catherine L. Gallagher MD[4,5], Sterling C. Johnson, PhD[1,2], Sanjay Asthana, MD[1,2,4], Bruce P. Hermann
PhD[1,2,5], Mark A. Sager MD[1,2], Kaj Blennow, MD[6-9], Henrik Zetterberg, MD[1,6,7,10-13], Cynthia M.
Carlsson, MD[1,2,5], Gwendlyn Kollmorgen, PhD[13], Clara Quijano-Rubio, PhD[14], Dane B. Cook, PhD[15, 16],
Dena B. Dubal, MD, PhD[17], Ozioma C. Okonkwo, PhD[1,2,*]

[1.] Wisconsin Alzheimer's Disease Research Center, Department of Medicine, School of Medicine and
Public Health, University of Wisconsin-Madison, Madison, WI, USA.
[2.] Wisconsin Alzheimer's Institute Madison, WI, USA.
[3.] NeuroGenomics and Informatics Center, Washington University School of Medicine, St. Louis, MO,
USA.
[4.] Geriatric Research Education and Clinical Center, William S. Middleton VA Hospital, Madison, WI,
USA.
[5.] Department of Neurology, School of Medicine and Public Health, University of Wisconsin-Madison,
Madison, WI, USA.
[6.] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska
Academy at the University of Gothenburg, Mölndal, Sweden.
[7.] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.
[8.] Paris Brain Institute, ICM, Pitié-Salpêtrière Hospital, Sorbonne University, Paris, France.
[9.] Neurodegenerative Disorder Research Center, Division of Life Sciences and Medicine, and
Department of Neurology, Institute on Aging and Brain Disorders, University of Science and
Technology of China and First Affiliated Hospital of USTC, Hefei, China.
[10.] Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK.
[11.] UK Dementia Research Institute at UCL, London, UK.
[12.] Hong Kong Center for Neurodegenerative Diseases, Clear Water Bay, Hong Kong, China.
[13.] Roche Diagnostics GmbH, Penzberg, Germany.
[14.] Roche Diagnostics International Ltd, Rotkreuz, Switzerland.
[15.] Research Service, William S. Middleton VA Hospital, Madison, WI, USA.
[16.] Department of Kinesiology, School of Education, University of Wisconsin-Madison, Madison, WI,
USA.
[17.] Department of Neurology and Weill Institute for Neurosciences, University of California, San
Francisco, California, USA.

*Correspondence:

Ira (Frahmand) Driscoll, PhD
University of Wisconsin - Madison
J5/M192 Clinical Science Center
600 Highland Avenue
Madison, WI 53792
Phone: (608) 262-5449
Email: idriscoll@medicine.wisc.edu

Ozioma Okonkwo, PhD
University of Wisconsin, Madison
J5/156M, Clinical Science Center
600 Highland Avenue
Madison, WI 53792
Phone: 608-265-4479
Email: ozioma@wisc.medicine.edu

Abstract

INTRODUCTION: While hallmarked by the accumulation of β-amyloid plaques (Aβ) and neurofibrillary tangles (tau) in the brain, Alzheimer's disease (AD) is a multifactorial disorder that involves additional pathological events, including neuroinflammation, neurodegeneration and synaptic dysfunction. AD-associated biomolecular changes seem to be attenuated in carriers of the functionally advantageous variant of the *KLOTHO* gene (KL-VS$_{HET}$). Independently, better cardiorespiratory fitness (CRF) is associated with better health outcomes, both in general and specifically with regard to AD pathology. Here we investigate whether the relationships between CRF (peak oxygen consumption (VO$_{2peak}$)) and cerebrospinal fluid (CSF) core AD biomarkers and those of neuroinflammation, neurodegeneration, and synaptic dysfunction differ for KL-VS$_{HET}$ compared to non-carriers (KL-VS$_{NC}$).

METHODS: The cohort, enriched for AD risk, consisted of cognitively unimpaired adults (N=136; Mean$_{AGE}$(SD)=62.5(6.7)) from the Wisconsin Registry for Alzheimer's Prevention and the Wisconsin Alzheimer's Disease Research Center. Covariate-adjusted (age, sex, parental AD history, *APOE*4+ status, and age difference between CSF sampling and exercise test) linear models examined the interaction between VO$_{2peak}$ and *KLOTHO* genotype on core AD biomarker levels in CSF [phosphorylated tau 181 (pTau$_{181}$), Aβ$_{42}$/Aβ$_{40}$, pTau$_{181}$/Aβ$_{42}$]. Analyses were repeated for CSF biomarkers of <u>neurodegeneration</u> [total tau (tTau), α-synuclein (α-syn), neurofilament light polypeptide (NfL)], <u>synaptic dysfunction</u> [neurogranin (Ng)], and <u>neuroinflammation</u> [glial fibrillary acidic protein (GFAP), soluble triggering receptor expressed in myeloid cells (sTREM2), chitinase-3-like protein 1 (YKL-40), interleukin 6 (IL-6), S100 calcium-binding protein B (S100B)].

RESULTS: The interaction between VO$_{2peak}$ and KL-VS$_{HET}$ was significant for **tTau** (*P*=0.05), **pTau$_{181}$** (*P*=0.03), **Ng** (*P*=0.02), **sTREM2** (*P*=0.03), and **YKL-40** (*P*=0.03), such that lower levels of each biomarker were observed for KL-VS$_{HET}$ who were more fit. No significant KL-VSxVO$_{2peak}$ interactions were observed for Aβ$_{42}$/Aβ$_{40}$, pTau$_{181}$/Aβ$_{42}$, α-syn, NfL, GFAP, IL-6 or S100B (all *P*s>0.09).

CONCLUSIONS: We report a synergistic relationship between KL-VS$_{HET}$ and CRF with regard to pTau$_{181}$, tTau, Ng, sTREM2 and YKL-40, suggesting a protective role for both KL-VS$_{HET}$ and better cardiovascular fitness against unfavorable AD-related changes. Their potentially shared biological mechanisms will require future investigations.

KEY WORDS: AD risk, Alzheimer's disease, exercise, protective factors, resilience, cerebrospinal fluid biomarkers

87

1   Research in Context

Systematic Review: PubMed literature review suggests that both *KLOTHO* KL-VS genotype and cardiorespiratory fitness (CRF) are associated with pathophysiological processes related to Alzheimer's Disease (AD). Both KL-VS heterozygotes (KL-VS$_{HET}$) and those with higher CRF fare better when faced with age-related biomolecular changes of relevance to AD. The present study investigates whether the relationships between CRF and cerebrospinal fluid biomarkers (CSF) of core AD neuropathology, neuroinflammation, neurodegeneration, and synaptic dysfunction differ for KL-VS$_{HET}$ compared to non-carriers.

Interpretation: Our findings suggest a synergistic relationship between KL-VS$_{HET}$ and higher CRF against core AD pathology along a range of unfavorable biomolecular changes implicated in this multifactorial disease. This supports the idea that CRF may interact with genetic factors to confer resilience against a multitude of adverse AD-associated processes.

Future Directions: Future studies should examine longitudinal changes in CSF biomarkers to determine whether maintaining or improving CRF over time enhances AD resilience in KL-VS$_{HET}$.

2   Introduction

Alzheimer's disease (AD), the most prevalent type of dementia, has garnered increasing attention as a pressing public health concern, particularly in light of the growing elderly population [1]. Given that age is the most significant risk factor for AD, this demographic shift is expected to lead to a marked rise in the number of individuals afflicted by this neurodegenerative disease [1]. Characterized by distinct pathophysiological hallmarks, the accumulation of beta-amyloid (Aβ) plaques and neurofibrillary tangles (tau) in the brain, AD causes irreparable cognitive and functional impairments [2]. This growing burden—affecting over 55 million people globally—highlights the critical need for advancing research efforts to develop preventative measures and therapeutic strategies to combat AD [1]. There is a growing interest in research surrounding modifiable and non-modifiable factors that might mitigate AD risk. Two such factors, a functionally advantageous *KLOTHO* KL-VS genotype (non-modifiable) and cardiorespiratory fitness (CRF; modifiable), are of particular interest as they are both associated with various favorable outcomes related to AD [3-12].

KLOTHO is considered an anti-aging and longevity gene that encodes klotho, a transmembrane protein responsible for regulating various aging processes in mammals [13, 14] . In humans, two genetic variants of *KLOTHO*, rs9536314 and rs9527025, combine to form a functional haplotype known as KL-VS [15]. The functionally advantageous KL-VS genotype (KL-VS$_{HET}$) is associated with higher circulating klotho protein levels and more favorable outcomes related to cardiovascular health, renal sufficiency, and cognitive function [14-17] within the context of aging. More recent literature examining *KLOTHO* in relation to AD suggests that KL-VS$_{HET}$ is associated with lower Aβ aggregation [11], tau burden [3, 4] and AD risk in apolipoprotein E (*APOE*) ε4 carriers [18]. Additionally, KL-VS$_{HET}$'s protective properties seem to extend to deleterious age-related changes in synaptic integrity, neurodegeneration, and neuroinflammatory responses [12]. This apparent broad neuroprotection suggests a critical role for KL-VS$_{HET}$ in safeguarding the brain from AD-associated biomolecular changes and related pathological events [3, 4, 11, 12].

CRF, a widely recognized index of habitual physical activity, is associated with reduced risk for AD-related dementia mortality [19], slower rates of gray matter atrophy in AD-relevant brain regions [5, 10], attenuation of white matter hyperintensities [20], and preservation of hippocampal volume with age [21]. Furthermore, emerging evidence suggests that physical exercise positively impacts not only cognition [5, 6, 10, 21] but also the underlying disease mechanisms, including Aβ deposition [7, 22, 23] and tau burden [24]. Additionally, higher CRF is also associated with less neuroinflammation [25] and better neuroplasticity [26]. The literature highlights the potential of CRF as a therapeutic strategy to address various pathological mechanisms of AD.

While both KL-VS$_{HET}$ and CRF seem to confer resilience against deleterious AD-associated changes, there is a notable absence of research that concurrently examines the synergy between these two factors with respect to their combined impact on biomolecular changes associated with AD. Moreover, existent interventions largely focus on mitigating Aβ and tau burden, even though AD is a multifactorial disease involving additional pathological processes, such as neuroinflammation, neurodegeneration, and synaptic dysfunction [27]. Hence, the objective of the current study is to investigate whether the relationships between CRF, indexed here by VO$_{2peak}$, and cerebrospinal fluid (CSF) biomarkers of core AD pathology, neurodegeneration, synaptic dysfunction, and neuroinflammation differ for *KLOTHO* KL-VS non-carriers (KL-VS$_{NC}$) and KL-VS$_{HET}$ in an older, cognitively unimpaired cohort enriched for

173 AD risk. We hypothesize that KL-VS$_{HET}$ individuals with higher CRF will have a more favorable profile
174 of investigated CSF biomarkers compared to KL-VS$_{NC}$.
175
176
177 **3   Methods**

178 3.1   Participants
179
180 The current sample is comprised of 136 (Mean$_{AGE}$(SD) = 62.5(6.7); 64% female) cognitively unimpaired,
181 middle-aged and older adults from the Wisconsin Registry for Alzheimer's Prevention (WRAP) [28] and
182 Wisconsin Alzheimer's Disease Research Center (WADRC) [11] who were genotyped for *APOE* and
183 *KLOTHO*, underwent CSF sampling, and had available VO$_{2peak}$ data. The cohort was enriched for
184 parental history of AD at enrollment, and subsequently has a higher prevalence of *APOE* ε4 allele
185 carriers (*APOE*4+) than what is observed in the general population. Cognitive normalcy was determined
186 by a standardized and multidisciplinary consensus based on performance on a comprehensive battery of
187 neuropsychological tests, lack of functional impairment, and absence of neurologic/psychiatric
188 conditions that might impair cognition [11, 28]. All study procedures were approved by the Institutional
189 Review Board at the University of Wisconsin and each participant provided written informed consent
190 before taking part.
191
192 3.2   Genotyping
193
194 Using the PUREGENE DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MN), DNA was isolated
195 from whole-blood samples. Ultraviolet spectrophotometry (DU 530 Spectrophotometer, Beckman
196 Coulter, Fullerton, CA) was used to measure DNA concentrations. Genotyping for *APOE* (rs429358 and
197 rs7412) and *KLOTHO* (rs9536314 for F352V and rs9527025 for C370S) was done by LGC Genomics
198 (Beverly, MA) via competitive allele-specific PCR-based KASP genotyping assays. Quality control
199 procedures have been previously published [11] and are considered acceptable. KL-VS homozygosity, a
200 rare genotype associated with lower klotho levels [16], was excluded from analyses due to sparse
201 sample size (N=6).
202
203 3.3   CSF Assessment
204
205 After a 12-hour fast, a lumbar puncture was conducted at L3-4 or L4-5 using a drip method and/or
206 gentle extraction technique into polypropylene syringes using a Sprotte 24- or 25-gauge spinal needle. A
207 thin needle was used to inject 1% lidocaine as a local anesthetic prior to the insertion of the Sprotte
208 spinal needle. 22 milliliters of CSF from each sample were pooled, gently mixed, and centrifuged at
209 2,000 g for 10 minutes. Supernatants were kept at 80°C and frozen in 0.5 milliliters aliquots in
210 polypropylene tubes.
211
212 Samples were analyzed for phosphorylated tau (pTau$_{181}$), Aβ$_{40,}$ and Aβ$_{42}$ to obtain biomarkers of core **AD**
213 **pathology** (pTau$_{181}$, Aβ$_{42}$/Aβ$_{40,}$ and pTau$_{181}$/Aβ$_{42}$), **neurodegeneration** [total tau (tTau), α-synuclein (α-
214 syn), neurofilament light polypeptide (NfL)], **synaptic dysfunction** [neurogranin (Ng)], and
215 **neuroinflammation** [glial fibrillary acidic protein (GFAP), soluble triggering receptor expressed in
216 myeloid cells 2 (sTREM2), chitinase-3-like protein 1 (YKL-40), interleukin 6 (IL-6), S100 calcium-
217 binding protein B (S100B)]) using the NeuroToolKit (NTK; Roche Diagnostics International Ltd,
218 Rotkruez, Switzerland). The NTK is a panel of exploratory prototype assays designed to robustly

219 evaluate established AD biomarkers (Aβ and tau) as well as emerging markers. This toolkit enables a
220 comprehensive characterization of AD pathology as well as a panel of synaptic, axonal, and glial
221 biomarkers, providing enhanced insights into the disease's pathophysiological processes [29].
222
223 3.4   Cardiorespiratory Fitness (VO_{2peak}) Assessment
224
225 Details of VO_{2peak} assessment have been previously published [6, 20]. To assess VO_{2peak}, the gold
226 standard for direct assessment of CRF [30], participants completed a maximal graded exercise test
227 administered by a certified exercise physiologist using standard operating procedures defined by
228 American College of Sports Medicine guidelines. Participants underwent medical screening, including a
229 resting ECG, to ensure safety before testing. Heart rate and rhythm were monitored continuously
230 alongside oxygen uptake (VO_2), carbon dioxide production, and other respiratory metrics using a
231 calibrated metabolic cart (TrueOne® 2400, Parvomedics). Participants walked at a self-selected speed,
232 with treadmill incline increasing 2.5% every two minutes until volitional exhaustion. Peak effort was
233 defined by at least two of the following criteria: (1) respiratory exchange ratio greater than or equal to
234 1.1, (2) achievement of 90% of age-predicted maximum heart rate, (3) perceived exertion greater than or
235 equal to 17, or (4) change in VO_2 less than 200 milliliters with an increase in work. Participants could
236 stop the test at any point, and recovery involved walking at 2 mph and 0% grade for five minutes. The
237 analyses were limited to participants who reached peak effort during graded exercise testing.
238
239 3.5   Statistical Analyses
240
241 All analyses were performed in R Statistical Software version 4.3.0 [31]. Demographic characteristics
242 were compared between KL-VS$_{NC}$ (N=93) and KL-VS$_{HET}$ (N=43) using independent-sample t-tests for
243 continuous measures and χ^2 tests for categorical measures. To examine whether the relationship between
244 VO_{2peak} and CSF biomarkers of core AD pathology, neurodegeneration, synaptic dysfunction, and
245 neuroinflammation differed between KL-VS$_{NC}$ and KL-VS$_{HET}$, we fitted a series of linear regression
246 models that incorporated a KL-VS* VO_{2peak} interaction term, while covarying for age, sex, age difference
247 between CSF sampling and exercise test, *APOE* ε4 status, and parental history of AD. If the interaction
248 was not significant, analyses were repeated after removing the KL-VS* VO_{2peak} term to examine the
249 main effects of KL-VS and VO_{2peak} to assess whether CSF biomarker levels differed by either factor
250 independently.
251
252
253 **4   Results**
254 4.1   Sample Characteristics
255
256 Table 1 details the background characteristics of the entire sample and by KL-VS genotype. The sample
257 was predominantly white (98%) and female (64%), with an average age of 62.5±6.7 years and enriched
258 for AD risk, with 38% carrying at least one *APOE* ε4 allele and 75% having a parental history of AD.
259 VO_{2peak} averaged 26.0±6.4 mL/kg/min. For the entire sample, the average age difference between CSF
260 sampling and the exercise test was 1.8±2.6 years. There were no significant differences in any of the
261 above-mentioned characteristics between KL-VS$_{NC}$ and KL-VS$_{HET}$ ($Ps \geq 0.17$).
262
263 4.2   CSF biomarkers of core AD pathology as a function of the *KLOTHO* KL-VS and VO_{2peak}
264

265 A significant interaction was observed between KL-VS genotype and VO_{2peak} for $pTau_{181}$ (P=0.03;
266 Table 2). Figure 1 illustrates this relationship, whereby $KL-VS_{HET}$ who were more fit had lower levels of
267 $pTau_{181}$. While KL-VSxVO_{2peak} interaction was not significant for $A\beta_{42}/A\beta_{40}$, the levels differed by KL-
268 VS genotype as indicated by a significant main effect of KL-VS (P=0.03). No significant interaction
269 (P=0.36) nor main effects of either KL-VS or VO_{2peak} were observed for $pTau_{181}/A\beta_{42}$ ($Ps \geq 0.07$).
270
271 4.3   CSF biomarkers of neurodegeneration, synaptic dysfunction and inflammation as a function of
272 the *KLOTHO* KL☐VS and VO_{2peak}
273
274 A significant KL-VSxVO_{2peak} interaction was observed for markers of neurodegeneration [**tTau**:
275 P=0.05], synaptic dysfunction [**Ng**: P=0.02] and neuroinflammation [Table 2; **sTREM2**: P=0.03; **YKL-
276 40**: P=0.03]. Figure 2A-D illustrates these findings; $KL-VS_{HET}$ with greater VO_{2peak} had lower levels of
277 tTau (A), Ng (B), YKL-40 (C) and sTREM2 (D). KL-VSxVO_{2peak} interaction was not significant for α-
278 syn, NfL, GFAP, S100B or IL-6 (all Ps>0.09). Additionally, no main effects of either KL-VS or VO_{2peak}
279 were significant for α-syn, NfL, GFAP or IL-6 (all Ps>0.10). S100B levels differed based on CRF, as
280 indicated by a significant main effect of VO_{2peak} (P=0.02).
281
282
283 **5   Discussion**
284 Our findings suggest that $KL-VS_{HET}$ in conjunction with higher CRF may offer protection against a
285 variety of adverse biomolecular changes associated with AD. Specifically, we report a synergistic
286 relationship between $KL-VS_{HET}$ and higher CRF with regard to core AD pathology ($pTau_{181}$),
287 neurodegeneration (tTau), synaptic dysfunction (Ng) and neuroinflammation (sTREM2 and YKL-40).
288 Together, our results contribute to the growing literature supporting the role for *KLOTHO* in modulating
289 age-related neuropathological processes and provide novel insights into how modifiable lifestyle factors,
290 CRF specifically, may interact with genetic factors to influence AD risk.
291
292 The significant interaction between $KL-VS_{HET}$ and CRF in relation to $pTau_{181}$ and tTau suggests a key
293 role for both fitness and genetic variation in mitigating tau-related pathology. Tau hyperphosphorylation
294 and aggregation are hallmark features of AD, contributing to neuronal dysfunction and cognitive decline
295 [27]. While CSF tTau and pTau are predictive markers of AD-related neurodegeneration and tangle
296 formation, they are not direct markers of these processes [32]. $pTau_{181}$ is also increasingly recognized as
297 more indicative of Aβ-related tau dysmetabolism [32]. Furthermore, we caution against the simplistic
298 interpretation of tTau as a marker of neurodegeneration given its nearly perfect correlation (~98%) with
299 $pTau_{181}$ across our center-wide data [29], which suggests that the two measures may be largely reflective
300 of overlapping rather than distinct pathological processes in our cognitively unimpaired cohort enriched
301 for AD risk. Based on extant literature, we know that individuals with higher plasma tau who engage in
302 more physical activity show a deceleration in cognitive decline [33]. Moreover, there is evidence that
303 $KL-VS_{HET}$ is associated with lower tau burden in aging adults [3, 4], suggesting that the $KL-VS_{HET}$
304 genotype may play a role in tau clearance or stabilization. Our study advances this understanding by
305 observing that, in $KL-VS_{HET}$, higher CRF is associated with lesser CSF pTau and tTau levels,
306 suggesting a combined role of genetic predisposition and physical activity in potentially slowing AD-
307 related tau pathology. This supports the notion that physical activity may exert genotype-specific
308 effects, providing greater neuroprotection in individuals with the $KL-VS_{HET}$ genotype. Thus, increasing
309 CRF through exercise may serve as a preventative therapeutic strategy for $KL-VS_{HET}$ individuals,
310 potentially mitigating AD-related tau pathology and its detrimental effects on cognitive health.

311
312 Ng is a postsynaptic protein expressed on postsynaptic spines of dendrites that is critical for synaptic
313 plasticity and memory formation [34]. Elevated Ng levels in CSF are increasingly recognized as a
314 marker of synaptic damage in AD [35]. Accumulating evidence suggests a protective effect of exercise
315 on synaptic health [26, 36, 37]. For instance, recent literature showed that higher levels of physical
316 activity are associated with lower Ng levels in individuals with low cardiovascular risk [36] and
317 enhanced synaptic plasticity through the upregulation of brain-derived neurotrophic factor (BDNF) [37].
318 Animal models have demonstrated that elevated circulating protein klotho levels enhances synaptic
319 plasticity and increases the expression of GluN2B, a key NMDA receptor subunit involved in synaptic
320 transmission [16, 17]. Our findings add to this body of research, showing that those with higher CRF
321 who concomitantly harbor the KL-VS$_{HET}$ genotype have lower CSF Ng levels, indicative of preserved
322 synaptic integrity. Furthermore, our results complement a recent study by our group, which reported that
323 KL-VS$_{HET}$ exhibit resilience against age-related increases in Ng levels [12]. While potentially protective
324 effects of KL-VS$_{HET}$ on synaptic health were present independent of fitness in our group's previous
325 work [12] our current findings suggest that higher CRF may amplify this neuroprotective effect,
326 highlighting a potential synergistic protective relationship between KL-VS$_{HET}$ and CRF against AD-
327 related synaptic dysfunction.
328
329 Neuroinflammation plays a central role in the progression of AD, contributing to neuronal injury and the
330 accumulation of amyloid and tau pathology [38, 39]. Two key biomarkers of neuroinflammation,
331 sTREM2 and YKL-40, reflect activity in microglia and astrocytes, respectively [40-42]. sTREM2 is a
332 soluble form of triggering receptor expressed in myeloid cells 2 (TREM2), which is predominantly
333 expressed on microglia, the immune cells of the central nervous system [43]. Upon microglial
334 activation, TREM2 signaling plays a crucial role in regulating microglial survival, proliferation, and
335 phagocytic activity in response to neuronal injury or the accumulation of pathological proteins such as
336 Aβ and tau. The release of sTREM2 into CSF reflects this activation process and is thought to act as a
337 modulator of neuroinflammation, amplifying protective microglial responses [43]. In AD, the elevation
338 of sTREM2 is seen across different stages of the disease, with the largest increase reported during the
339 mild cognitive impairment (MCI) phase before transitioning to AD [44]. Moreover, CSF sTREM2
340 levels are significantly elevated in individuals with AD compared to healthy controls [45]. This suggests
341 that microglial activation intensifies early in the disease process and persists throughout its progression,
342 making sTREM2 a useful biomarker for tracking neuroinflammation within the context of
343 neurodegeneration.
344
345 Similarly, YKL-40, a glycoprotein primarily expressed by astrocytes, acts as an early indicator of
346 neuroinflammation and disease progression [42]. Growing evidence suggests that YKL-40 is also a
347 promising biomarker for glial inflammatory response in AD, with significantly elevated CSF levels
348 reported in individuals with AD compared to those cognitively unimpaired [42, 46], and also higher
349 levels in *APOE* ε4 carriers with MCI [47]. Exercise modulates these neuroinflammatory markers in AD;
350 CSF sTREM2 levels increase following physical activity, which may reflect transient microglial
351 activation in response to acute exercise in individuals with AD [48]. However, it is important to note
352 that individuals with AD typically exhibit elevated baseline CSF sTREM2 due to disease-related
353 microglial activation [45], which may amplify the observed acute response. In contrast, our findings
354 demonstrate that, in KL-VS$_{HET}$, higher CRF is associated with lower CSF concentrations of both
355 sTREM2 and YKL-40. This contrast suggests that while acute exercise may transiently activate
356 microglia in individuals with AD, habitual exercise, reflected by higher CRF, may confer long-term

neuroprotective effects by reducing chronic neuroinflammation in otherwise healthy populations. This implies a synergistic benefit of KL-VS$_{HET}$ and physical fitness, suggesting that exercise could offer targeted benefits in reducing AD-related neuroinflammation in certain populations. Additionally, in the present study, higher CRF was associated with elevated S100B levels regardless of KL-VS genotype, contrary to what would be expected based on the literature. Although S100B is generally linked to inflammatory processes [49], its upregulation in individuals with higher CRF could indicate an adaptive response, promoting repair and neurotrophic effects in the brain rather than solely reflecting pathological inflammation. Further investigation is needed to clarify the role of S100B within the context of fitness and neurodegeneration. Overall, our findings reinforce the idea that klotho, which is inherently higher in KL-VS$_{HET}$, and CRF might interact to mitigate neuroinflammatory processes in AD, providing a promising approach for slowing disease progression.

No significant interactions were observed for Aβ-related biomarkers. This is noteworthy given that the literature suggests that tau pathology might be more responsive to KL-VS heterozygosity compared to Aβ accumulation [4]. Moreover, KL-VS-related effects on Aβ are more pronounced in individuals carrying the *APOE* ε4 allele [13], potentially explaining the absence of significant findings related to Aβ in our sample. Further studies are needed to elucidate whether longitudinal fitness interventions yield stronger effects on these biomarkers in KL-VS$_{HET}$, especially in *APOE* ε4-positive individuals. Together, the evidence underscores the complexity of genetic and environmental interplay in modulating AD-related biomolecular changes and emphasizes the importance of refining intervention strategies to target specific pathways and patient subgroups effectively [50].

This study is not without limitations. The cohort is predominantly white and well-educated, with a higher proportion of participants with parental history of AD or carrying at least one *APOE* ε4 allele compared to the general population. These characteristics may limit the generalizability of our results. Furthermore, as this is a cross-sectional study, we cannot assess the effects of the exposures on changes in CSF biomarker levels over time. Another potential limitation is the time lag between the lumbar puncture and the maximal graded exercise test; we did statistically adjust for this differential by incorporating the time lag as a covariate in the model. Notwithstanding the limitations, we believe this work makes a clinically meaningful contribution to the literature. Furthermore, WRAP and WADRC are ongoing longitudinal studies that continue to collect data and have also recently increased efforts to enroll participants from groups historically under-represented in research. Thus, future investigations will be able to examine how the interaction between KL-VS$_{HET}$ and VO$_{2peak}$ relates to prospective changes in the biomarkers examined in this study in larger, more diverse samples.

Overall, these findings suggest a positive synergy between KL-VS$_{HET}$ and better CRF. While the precise mechanisms by which the KL-VS$_{HET}$ genotype or CRF exerts protective effects are not entirely clear, a better understanding of lifestyle interventions aimed at improving fitness levels and genetic variants that modify AD risk hold promise to provide new avenues for prevention or treatment.

Conflict of Interest Statement

All authors have no conflict of interest directly related to this study.

S.C.J. has served on advisory boards for ALZPath and Enigma Biosciences. **K.B.** has served as a consultant, on advisory boards, or on data monitoring committees for Acumen, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; has served at data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, which is a part of the GU Ventures Incubator Program (outside submitted work). **H.Z.** has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). **G.K.** is a full-time employee of Roche Diagnostics GmbH. **C. Q.-R.** is a full-time employee of Roche Diagnostics International Ltd. **D.B.D**. has consulted for Unity Biotechnology and S.V. Health Investors. All other authors have no relevant disclosures to report.

Disclosures

Klotho is the subject of an international patent issued and held by the Regents of the University of California.

The NeuroToolKit is a panel of exploratory prototype assays designed to robustly evaluate biomarkers associated with key pathologic events characteristic of AD and other neurological disorders, used for research purposes only and not approved for clinical use (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Elecsys β-amyloid (1–42) and Elecsys Phospho-Tau (181P) CSF assays are approved for clinical use. COBAS and ELECSYS are trademarks of Roche. All other product names and trademarks are the property of their respective owners.

References

1. *2024 Alzheimer's disease facts and figures.* Alzheimers Dement, 2024. **20**(5): p. 3708-3821.
2. Van Cauwenberghe, C., C. Van Broeckhoven, and K. Sleegers, *The genetic landscape of Alzheimer disease: clinical implications and perspectives.* Genet Med, 2016. **18**(5): p. 421-30.
3. Driscoll, I., et al., *AD-associated CSF biomolecular changes are attenuated in KL-VS heterozygotes.* Alzheimers Dement (Amst), 2022. **14**(1): p. e12383.
4. Driscoll, I., et al., *Age-Related Tau Burden and Cognitive Deficits Are Attenuated in KLOTHO KL-VS Heterozygotes.* J Alzheimers Dis, 2021. **79**(3): p. 1297-1305.
5. Boots, E.A., et al., *Cardiorespiratory fitness is associated with brain structure, cognition, and mood in a middle-aged cohort at risk for Alzheimer's disease.* Brain Imaging Behav, 2015. **9**(3): p. 639-49.
6. Vesperman, C.J., et al., *Cardiorespiratory fitness and cognition in persons at risk for Alzheimer's disease.* Alzheimers Dement (Amst), 2022. **14**(1): p. e12330.
7. Okonkwo, O.C., et al., *Physical activity attenuates age-related biomarker alterations in preclinical AD.* Neurology, 2014. **83**(19): p. 1753-60.
8. Okonkwo, O. and H. van Praag, *Exercise Effects on Cognitive Function in Humans.* Brain Plast, 2019. **5**(1): p. 1-2.
9. Gaitán, J.M., et al., *Effects of Aerobic Exercise Training on Systemic Biomarkers and Cognition in Late Middle-Aged Adults at Risk for Alzheimer's Disease.* Front Endocrinol (Lausanne), 2021. **12**: p. 660181.
10. Dougherty, R.J., et al., *Cardiorespiratory fitness mitigates brain atrophy and cognitive decline in adults at risk for Alzheimer's disease.* Alzheimers Dement (Amst), 2021. **13**(1): p. e12212.
11. Erickson, C.M., et al., *KLOTHO heterozygosity attenuates APOE4-related amyloid burden in preclinical AD.* Neurology, 2019. **92**(16): p. e1878-e1889.
12. Driscoll, I.F., et al., *KLOTHO KL-VS heterozygosity is associated with diminished age-related neuroinflammation, neurodegeneration, and synaptic dysfunction in older cognitively unimpaired adults.* Alzheimers Dement, 2024. **20**(8): p. 5347-5356.
13. Kurosu, H., et al., *Suppression of aging in mice by the hormone Klotho.* Science, 2005. **309**(5742): p. 1829-33.
14. Buchanan, S., et al., *Klotho, Aging, and the Failing Kidney.* (1664-2392 (Print)).
15. Arking, D.E., et al., *Association of human aging with a functional variant of klotho.* Proc Natl Acad Sci U S A, 2002. **99**(2): p. 856-61.
16. Dubal, D.B., et al., *Life extension factor klotho enhances cognition.* Cell Rep, 2014. **7**(4): p. 1065-76.
17. Dubal, D.B., et al., *Life extension factor klotho prevents mortality and enhances cognition in hAPP transgenic mice.* J Neurosci, 2015. **35**(6): p. 2358-71.
18. Belloy, M.E., et al., *Association of Klotho-VS Heterozygosity With Risk of Alzheimer Disease in Individuals Who Carry APOE4.* JAMA Neurol, 2020. **77**(7): p. 849-862.
19. LIU, R., et al., *Cardiorespiratory Fitness as a Predictor of Dementia Mortality in Men and Women.* Medicine & Science in Sports & Exercise, 2012. **44**(2): p. 253-259.
20. Vesperman, C.J., et al., *Cardiorespiratory fitness attenuates age-associated aggregation of white matter hyperintensities in an at-risk cohort.* Alzheimers Res Ther, 2018. **10**(1): p. 97.

531   21.   Dougherty, R.J., et al., *Relationships between cardiorespiratory fitness, hippocampal volume,*
532   *and episodic memory in a population at risk for Alzheimer's disease.* Brain Behav, 2017. **7**(3): p.
533   e00625.
534   22.   Law, L.L., et al., *Moderate intensity physical activity associates with CSF biomarkers in a cohort*
535   *at risk for Alzheimer's disease.* Alzheimers Dement (Amst), 2018. **10**: p. 188-195.
536   23.   Liang, K.Y., et al., *Exercise and Alzheimer's disease biomarkers in cognitively normal older*
537   *adults.* Ann Neurol, 2010. **68**(3): p. 311-8.
538   24.   Brown, B.M., et al., *Self-Reported Physical Activity is Associated with Tau Burden Measured by*
539   *Positron Emission Tomography.* J Alzheimers Dis, 2018. **63**(4): p. 1299-1305.
540   25.   Wang, M., et al., *Exercise suppresses neuroinflammation for alleviating Alzheimer's disease.* J
541   Neuroinflammation, 2023. **20**(1): p. 76.
542   26.   de Sousa Fernandes, M.S., et al., *Effects of Physical Exercise on Neuroplasticity and Brain*
543   *Function: A Systematic Review in Human and Animal Studies.* Neural Plast, 2020. **2020**: p.
544   8856621.
545   27.   Masters, C.L., et al., *Alzheimer's disease.* Nat Rev Dis Primers, 2015. **1**: p. 15056.
546   28.   Johnson, S.C., et al., *The Wisconsin Registry for Alzheimer's Prevention: A review of findings and*
547   *current directions.* Alzheimers Dement (Amst), 2018. **10**: p. 130-142.
548   29.   Van Hulle, C., et al., *An examination of a novel multipanel of CSF biomarkers in the Alzheimer's*
549   *disease clinical and pathological continuum.* Alzheimers Dement, 2021. **17**(3): p. 431-445.
550   30.   Medicine, A.C.o.S., *ACSM's guidelines for exercise testing and prescription.* 2013: Lippincott
551   williams & wilkins.
552   31.   R Development Core Team, *R: A language and environment for statistical computing.* 2021, R
553   Foundation for Statistical Computing: Vienna, Austria.
554   32.   Zetterberg, H. and K. Blennow, *Moving fluid biomarkers for Alzheimer's disease from research*
555   *tools to routine clinical diagnostics.* Mol Neurodegener, 2021. **16**(1): p. 10.
556   33.   Desai, P., et al., *Longitudinal Association of Total Tau Concentrations and Physical Activity With*
557   *Cognitive Decline in a Population Sample.* JAMA Netw Open, 2021. **4**(8): p. e2120398.
558   34.   Gerendasy, D.D. and J.G. Sutcliffe, *RC3/neurogranin, a postsynaptic calpacitin for setting the*
559   *response threshold to calcium influxes.* Mol Neurobiol, 1997. **15**(2): p. 131-63.
560   35.   Kester, M.I., et al., *Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in*
561   *Symptomatic Alzheimer Disease.* JAMA Neurol, 2015. **72**(11): p. 1275-80.
562   36.   Stojanovic, M., et al., *Effect of exercise engagement and cardiovascular risk on neuronal injury.*
563   Alzheimers Dement, 2023. **19**(10): p. 4454-4462.
564   37.   Lu, Y., et al., *Recent advances on the molecular mechanisms of exercise-induced improvements*
565   *of cognitive dysfunction.* Transl Neurodegener, 2023. **12**(1): p. 9.
566   38.   Kinney, J.W., et al., *Inflammation as a central mechanism in Alzheimer's disease.* Alzheimers
567   Dement (N Y), 2018. **4**: p. 575-590.
568   39.   Leng, F. and P. Edison, *Neuroinflammation and microglial activation in Alzheimer disease:*
569   *where do we go from here?* Nat Rev Neurol, 2021. **17**(3): p. 157-172.
570   40.   Suárez-Calvet, M., et al., *Early increase of CSF sTREM2 in Alzheimer's disease is associated with*
571   *tau related-neurodegeneration but not with amyloid-β pathology.* Mol Neurodegener, 2019.
572   **14**(1): p. 1.
573   41.   Nordengen, K., et al., *Glial activation and inflammation along the Alzheimer's disease*
574   *continuum.* Journal of Neuroinflammation, 2019. **16**(1): p. 46.

575 42. Connolly, K., et al., *Potential role of chitinase-3-like protein 1 (CHI3L1/YKL-40) in*
576   *neurodegeneration and Alzheimer's disease.* Alzheimers Dement, 2023. **19**(1): p. 9-24.
577 43. Suárez-Calvet, M., et al., *sTREM2 cerebrospinal fluid levels are a potential biomarker for*
578   *microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers.*
579   EMBO Mol Med, 2016. **8**(5): p. 466-76.
580 44. Hok-A-Hin, Y.S., et al., *Neuroinflammatory CSF biomarkers MIF, sTREM1, and sTREM2 show*
581   *dynamic expression profiles in Alzheimer's disease.* Journal of Neuroinflammation, 2023. **20**(1):
582   p. 107.
583 45. Heslegrave, A., et al., *Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's*
584   *disease.* Mol Neurodegener, 2016. **11**: p. 3.
585 46. Mavroudis, I., et al., *YKL-40 as a Potential Biomarker for the Differential Diagnosis of*
586   *Alzheimer's Disease.* Medicina (Kaunas), 2021. **58**(1).
587 47. Wang, L., et al., *Cerebrospinal fluid levels of YKL-40 in prodromal Alzheimer's disease.* Neurosci
588   Lett, 2020. **715**: p. 134658.
589 48. Jensen, C.S., et al., *Exercise as a potential modulator of inflammation in patients with*
590   *Alzheimer's disease measured in cerebrospinal fluid and plasma.* Exp Gerontol, 2019. **121**: p. 91-
591   98.
592 49. Donato, R., *S100: a multigenic family of calcium-modulated proteins of the EF-hand type with*
593   *intracellular and extracellular functional roles.* Int J Biochem Cell Biol, 2001. **33**(7): p. 637-68.
594 50. Sperling, R.A., C.R. Jack, Jr., and P.S. Aisen, *Testing the right target and right drug at the right*
595   *stage.* Sci Transl Med, 2011. **3**(111): p. 111cm33.
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621

622 **TABLES**

623 **Table 1.** Background characteristics of study participants.

	Entire Sample (N = 136)	KL-VS$_{NC}$ (N = 93)	KL-VS$_{HET}$ (N = 43)	P
Age at CSF Mean (SD)	62.5 (6.7)	62.8 (6.7)	62.4 (6.7)	0.73
Female N (%)	87 (64)	61 (66)	26 (61)	0.83
White N (%)	133 (98)	91 (98)	42 (98)	0.38
APOE4+ N (%)	52 (38)	36 (39)	16 (37)	0.78
Parental History of AD N (%)	102 (75)	71 (76)	31 (72)	0.78
Age$_{exercise}$ – Age$_{LP}$ Mean (SD)	1.8 (2.6)	1.5 (2.5)	2.3 (2.9)	.17
VO$_{2peak}$ (mL/kg/min) Mean (SD)	26.0 (6.4)	26.2 (6.6)	25.7 (5.9)	0.57

624 ***Abbreviations:** AD = Alzheimer's disease; Age$_{LP}$ = age at lumbar puncture; Age$_{exercise}$ = age at the exercise test visit;
625 *APOE*4+ = carrier of at least one *APOE* ε4 allele; CRF = cardiorespiratory fitness; CSF = cerebrospinal fluid; KL-
626 VS$_{HET}$ = *KLOTHO* KL-VS heterozygote; KL-VS$_{NC}$ =*KLOTHO* KL-VS non-carrier; mL/kg/min = milliliters per kilogram per
627 minute; SD = standard deviation; VO$_{2peak}$ = peak oxygen consumption.
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650

Table 2. CSF biomarkers of core AD neuropathology, neurodegeneration, synaptic dysfunction, and neuroinflammation as a function of the *KLOTHO* KL☐VS and VO_{2peak}.

AD Pathology	Model		β	S.E.	*P*
$A\beta_{42}/A\beta_{40}$	Main Effect*	$KL\text{-}VS_{HET}$	-0.01	0.01	**0.03**
	Main Effect*	VO_{2peak}	-0.002	0.002	0.50
	Interaction	$KL\text{-}VS_{HET}$ x VO_{2peak}	---	---	---
$pTau_{181}/A\beta_{42}$	Main Effect*	$KL\text{-}VS_{HET}$	0.21	0.40	0.60
	Main Effect*	VO_{2peak}	0.004	0.01	0.61
	Interaction	$KL\text{-}VS_{HET}$ x VO_{2peak}	---	---	---
$pTau_{181}$	Main Effect	$KL\text{-}VS_{HET}$	---	---	---
	Main Effect	VO_{2peak}	---	---	---
	Interaction**	$KL\text{-}VS_{HET}$ x VO_{2peak}	-0.02	0.01	**0.03**
Neurodegeneration					
α-Synuclein	Main Effect*	$KL\text{-}VS_{HET}$	0.58	0.35	0.10
	Main Effect*	VO_{2peak}	0.001	0.01	0.91
	Interaction	$KL\text{-}VS_{HET}$ x VO_{2peak}	---	---	---
NfL	Main Effect*	$KL\text{-}VS_{HET}$	0.05	0.31	0.54
	Main Effect*	VO_{2peak}	-0.001	0.01	0.88
	Interaction	$KL\text{-}VS_{HET}$ x VO_{2peak}	---	---	---
tTau	Main Effect	$KL\text{-}VS_{HET}$	---	---	---
	Main Effect	VO_{2peak}	---	---	---
	Interaction**	$KL\text{-}VS_{HET}$ x VO_{2peak}	-0.02	0.01	**0.05**
Synaptic Dysfunction					
Neurogranin	Main Effect	$KL\text{-}VS_{HET}$	---	---	---
	Main Effect	VO_{2peak}	---	---	---
	Interaction**	$KL\text{-}VS_{HET}$ x VO_{2peak}	-0.03	0.01	**0.02**
Neuroinflammation					
GFAP	Main Effect*	$KL\text{-}VS_{HET}$	0.34	0.24	0.16
	Main Effect*	VO_{2peak}	-0.002	0.01	0.71
	Interaction	$KL\text{-}VS_{HET}$ x VO_{2peak}	---	---	---
sTREM2	Main Effect	$KL\text{-}VS_{HET}$	---	---	---
	Main Effect	VO_{2peak}	---	---	---
	Interaction**	$KL\text{-}VS_{HET}$ x VO_{2peak}	-0.02	0.01	**0.03**
YKL-40	Main Effect	$KL\text{-}VS_{HET}$	---	---	---
	Main Effect	VO_{2peak}	---	---	---
	Interaction**	$KL\text{-}VS_{HET}$ x VO_{2peak}	-0.02	0.008	**0.03**
S100B	Main Effect*	$KL\text{-}VS_{HET}$	0.15	0.20	0.47
	Main Effect*	VO_{2peak}	0.01	0.005	**0.02**
	Interaction	$KL\text{-}VS_{HET}$ x VO_{2peak}	---	---	---
IL-6	Main Effect*	$KL\text{-}VS_{HET}$	0.53	0.35	0.13
	Main Effect*	VO_{2peak}	0.004	0.01	0.61
	Interaction	$KL\text{-}VS_{HET}$ x VO_{2peak}	---	---	---

Abbreviations: $A\beta_{42}/A\beta_{40}$ = β-amyloid 42/40; CSF = cerebrospinal fluid; GFAP = glial fibrillary acidic protein; KL-VS_{HET} = *KLOTHO* KL-VS heterozygotes; KL-VS_{NC} = *KLOTHO* KL-VS non-carriers; NfL = neurofilament light chain; $pTau_{181}$ = phosphorylated tau 181; S.E. = standard error; sTREM2 = soluble triggering receptor expressed on myeloid cells 2; tTau = total tau; VO_{2peak} = peak oxygen consumption; YKL-40 = chitinase-3-like protein

659   "---" in the tables denotes a non-significant interaction or main effect.

660   **Interaction: Results of linear regression model of KL-VS, VO_{2peak}, and KL-VS$_{HET}$xVO_{2peak} interaction on CSF biomarker.

661 Models were adjusted for age, sex, age difference between CSF sampling and exercise test, *APOE* ε4 status, and parental

662 history of AD.

663 *Main Effect: Results of linear regression model of KL-VS and VO_{2peak} on CSF biomarker, excluding KL-VS$_{HET}$xVO_{2peak}

664 interaction when the interaction was not significant. Models were adjusted for age, sex, age difference between CSF

665 sampling and exercise test, *APOE* ε4 status, and parental history of AD.

666

667

668

669

670

671

672

673

674

675

676

677

678

679

680

681

682

683

684

685

686

687

688

689

690

691

692

693

694

695

696

697

698

699

700

701

702

703

704

705

706

707   **FIGURES**
708
709   **Figure 1. KL-VS genotype differences in CSF pTau$_{181}$ based on CRF (VO$_{2peak}$).** KL-VS$_{HET}$ with
710   high CRF had lower levels of pTau$_{181}$.
711

AD Pathology
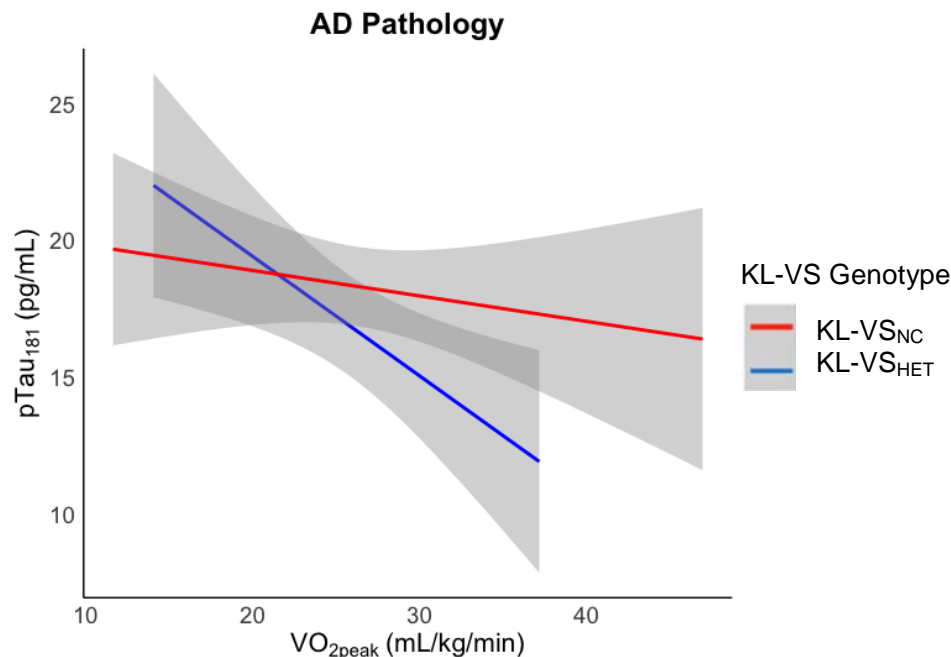
712
713   *Abbreviations:* AD = Alzheimer's Disease; kg = kilogram; KL-VS$_{HET}$ = *KLOTHO* KL-VS heterozygote; KL-
714   VS$_{NC}$ =*KLOTHO* KL-VS non-carrier; min = minute; mL = milliliter; pg = picograms; pTau$_{181}$ = phosphorylated tau 181;
715   VO$_{2peak}$ = peak oxygen consumption.
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737

**Figure 2. KL-VS differences in CSF A) tTau, B) Ng, C) YKL-40, and D) sTREM2 based on CRF
(VO_{2peak}).** KL-VS$_{HET}$ with high CRF had lower levels of neurodegeneration (tTau), synaptic dysfunction
(Neurogranin) and neuroinflammation (sTREM2 and YKL-40).
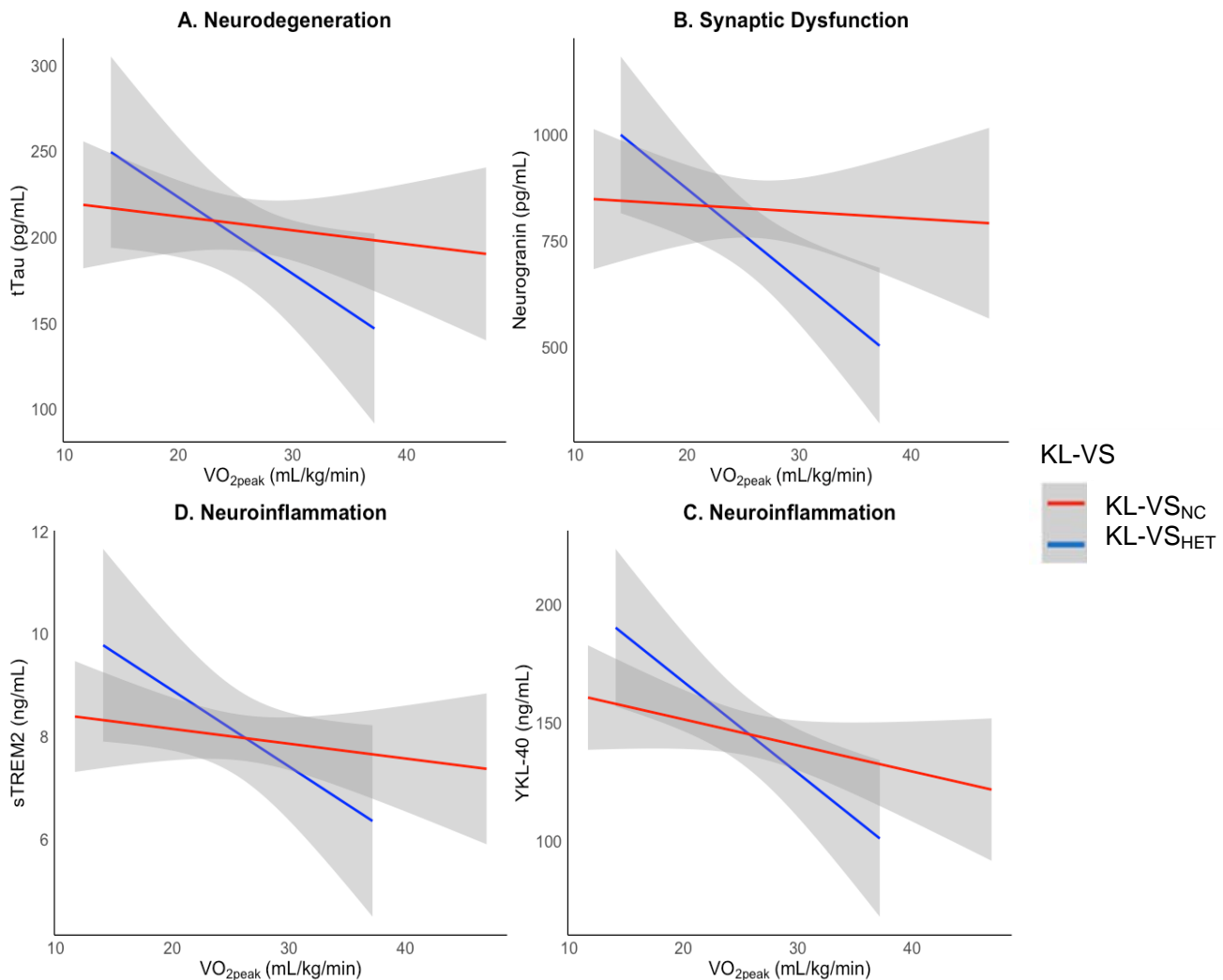
Abbreviations: KL-VS$_{HET}$ = *KLOTHO* KL-VS heterozygote; KL-VS$_{NC}$ =*KLOTHO* KL-VS non-carrier; kg = kilogram; min =
minute ; mL = milliliter; ng = nanogram; pg = picogram; sTREM2 = soluble triggering receptor expressed on myeloid cells
2; tTau = total tau; VO_{2peak} = peak oxygen consumption; YKL-40 = chitinase-3-like protein 1.