Food for Thought: Can Intermittent Fasting Induced Ketosis Ameliorate Dementia by Decreasing Tau Hyperphosphorylation and Neuroinflammation in *Drosophila melanogaster?*

ABSTRACT:

Background: Dementia is highly prevalent neuropsychiatric condition characterized by impairments in memory, reasoning, mood, and behavior. Yet it has no effective treatment that targets the underlying biochemical pathology. The main goals of this project were to utilize a *Drosophila melanogaster* model of Dementia and determine whether ketosis induced by intermittent time-restricted feeding (iTRF) can lead to decreased mortality, improved memory, and enhanced locomotor function. **Methods:** flies with the V337M Tau mutation were used as a validated model of dementia. Both demented and control flies were subjected to either: (a) 24 hours access to food/ad libitum or ad lib OR (b) a Time Restricted Feeding (TRF) schedule with 10 hours of fasting during lights off and 14 hours of access to food, mostly during lights on. To assess the effect on lifespan, the percent of flies surviving over time, within each group, was calculated. Impaired geotaxis, diminished climbing motivation and ability, was assessed as a measure of locomotor ability. The Aversive phototaxic suppression assay was used to assess learning/memory; flies learned to avoid light that is paired with an aversive stimulus. Group differences were analyzed with survival curves. Chi-square tests were used for the categorical variables. **Results:** Survival curve analysis showed that the flies subjected to iTRF for 4 days lived longer than flies who fed ad lib, with the effect being more pronounced among the dementia group. Flies with Dementia had impaired climbing ability compared with controls, but within each group, there was no significant difference in locomotor function, among those fed ad lib versus iTRF. Flies with dementia demonstrated significant impairment in short-term memory and learning compared to the control flies (p-value = 0.03). iTRF did not improve short-term memory among control flies (p-value = 0.55) but greatly enhanced memory and learning in the dementia group (p-value = 0.04). **Conclusion:** Intermittent Time Restricted Feeding, and associated ketosis, may be a promising new intervention for dementia. Future studies should evaluate the underlying metabolic pathways as well as the changes in brain pathology that accompany improvement in clinical symptoms.

INTRODUCTION:

Dementia is a devastating neuropsychiatric condition characterized by impairments in memory, reasoning, mood, and behavior; the decline in mental status is typically significant enough to preclude daily independent functioning (Gale, Acar, Daffner., 2018). The most common forms of dementia are seen in Alzheimer's disease (AD) for adults over age 65, and frontotemporal dementia (FTD) among individuals younger than 65 years (Rathnavalli, 2002). Based on World Health Organization estimates, more than 55.2 million people worldwide currently suffer from dementia, with approximately 7 million new cases per year, and an anticpated increase to 139 million in 2050 (Dementia, 2021). Despite the prevalence and impact of dementia, it has no effective treatment besides some drugs that can delay symptom progression. No treatment strategy targets the underlying biochemical pathology (Poudel and Park, 2022).

On a cellular level, neuronal communication in dementias is affected by two main culprits – tau and amyloid proteins. In healthy neurons, tau is found within the axons where it binds to microtubules, stabilizing them and maintaining normal neuronal architecture. A high degree of phosphorylation results in tau detachment from microtubules and subsequent tau aggregation, finally causing the formation of neurofibrillary tangles (Iqbal et al., 2010). Amyloid which starts out as amyloid precursor protein can be processed to produce either a healthy soluble protein or one that is toxic (amyloid-beta or Aβ) which clumps and accumulates into amyloid plaques. Together, extracellular amyloid plaques and intracellular neurofibrillary tangles, represent the classic neuropathological hallmarks of AD. Finally, oxidative stress, mitochondrial dysfunction, and neuroinflammation all have an additive effect in contributing to the ultimate disease presentation of AD (Pugazhenthi et al., 2016).

Recent research suggests that in AD and FTD, beta amyloid clusters precede abnormal tau. Once amyloid-beta has accumulated to a certain level it leads to the production of more amyloid-beta and more abnormal tau and latter is associated with clinical symptoms (Jack et al., 2019). Also, reduction of Tau protein levels leads to an amelioration of Aβ-induced learning and memory impairment (Robertson et al., 2007). Tau pathology in various forms is also seen in a host of other neurodegenerative conditions characterized by dementia and movement disorders, including chronic traumatic encephalopathy or CTE (Zhang et al 2022). Given the key role it

plays in most dementias and its association with onset of clinical symptoms (figure 1), Tau is an attractive target for prevention and therapy of such neurodegenerative disorders.

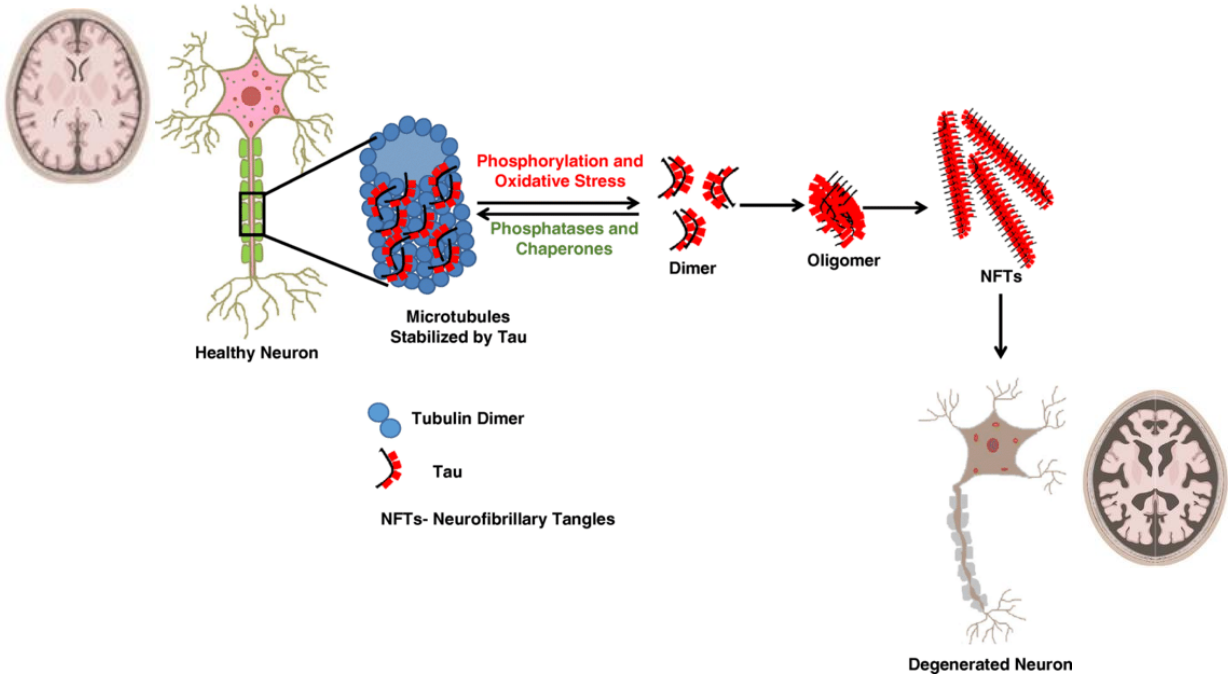


Figure 1. Tau Hyperphosphorylation pathway for neurodegeneration (modified from Nalini et al, 2018, Journal of Molecular Neuroscience)

While many new drugs for dementia are under investigation, none of the existing drugs works to prevent or modify the cerebral pathology of dementia. Also, effective drug delivery to the brain is hindered by the blood-brain-barrier, which is a network of closely spaced cells and blood vessels which prevent most substances from reaching the brain (Daneman and Pratt, 2015). Nutrition has been proposed a key modifiable factor in the progression of dementia and many pathophysiologic mechanisms connect glucose metabolism to Alzheimer's disease, where hyperglycemia is associated with neuroinflammation oxidative stress which can trigger the amyloid cascade of AD (Taylor et.al. 2017). Thus, the ketogenic diet and ketone body supplementation have been proposed as potential therapeutic options for Alzheimer's disease. The ketogenic diet is a high saturated fat/low carbohydrate diet switches the body from glucose metabolism to fat burning and producing ketone bodies as an alternative fuel to glucose (Dowis and Banga, 2021). Ketosis can be achieved through either fasting or carbohydrate restriction and ketone bodies have been shown to have beneficial effects on neurons by decreasing hyperexcitability and inflammation (Hertz et al., 2015). Recent studies have indicated that

intermittent fasting, which is less restrictive than ketogenic diets, may protect against neurodegeneration observed in animal models of Parkinson's disease, Huntington's disease, and traumatic brain injury (TBI) following dietary intermittent protocols but little is known of the outcome in Alzheimer's and Frontotemporal dementia (Nasiruddin et al., 2020).

Drosophila melanogaster or the common fruit fly is a convenient and efficient model of neurodegenerative disease and flies shave been used successfully to dissect the genetic basis of complex behaviors such as sleep, learning, and memory (Lessing and Bonini, 2009). Previous studies have clearly shown that the expression of dementia-related gene products (tau protein and Aβ42 peptide, respectively) causes expected phenotypes in flies (Bonner and Boulianne, 2011). Like AD patients, flies show a predictable decline of neurons upon Aβ42 and/or Tau overexpression and the associated deficits in cognition and behavior can be analyzed using validated assays (Prubing, Voigt, and Shulz, 2013).

Main Objectives

To use a *Drosophila melanogaster* model of dementia and determine whether ketosis induced by intermittent fasting or time-restricted feeding (TRF) can lead to:

1. Decreased mortality or a measurable positive impact on lifespan.
2. Improved locomotor function measure by the negative geotaxis assay
3. Enhanced cognitive function demonstrated by improved learning in the aversive conditioning task.

METHODS/MATERIALS:

Fly Stocks were obtained from Bloomington Stock Center (Bloomington, Indiana) and raised at 23^{o} C on standard cornmeal-molasses medium. Flies for the experimental group (w[1118]; P{w[+mC]=GMR-MAPT.V337M}10/TM3, Sb[1]) – expressed human microtubule-associated protein tau (MAPT) with amino acid change V337M and the control group (w[1118]) did not contain abnormal tau.

Mutations in the microtubule-associated protein tau (MAPT) gene, in humans, causes deposition of hyperphosphorylated tau protein in neurons and glia and are associated with inherited frontotemporal dementia (FTD) (Spillantini et al., 1998). The V337M MAPT mutation,

in particular, has been associated with FTD with severe frontotemporal and limbic degeneration and cognitive deficits (Sumi SM et al., 1992). Furthermore, the structure and composition of tau aggregates associated with this mutation are similar to those found in neurofibrillary tangles in Alzheimer disease (AD) (Spillantini et al., 1996). Thus, flies with the V337M MAPT mutation offer a robust model of tauopathy associated dementias, including Alzheimer's Disease and Frontotemporal Dementia (Spina et al., 2017).

Intermittent fasting or time-restricted feeding is gaining popularity and scientific support as a means to attenuate aging related processing, in both flies (Gill et al., 2015) and humans (de Cabo R and Mattson MP, 2019) Recent research investigating various intermittent time-restricted feeding (iTRF) regimens (Ulgherait et al., 202) was used to derive an iTRF regimen for this study, taking into consideration the fact that flies with neurodegenerative disease may be more vulnerable to fasting states. A fasting window of 10 hours overnight (8 PM- 6 AM), for a period of 4 days was found to be optimal based on initial experiments.

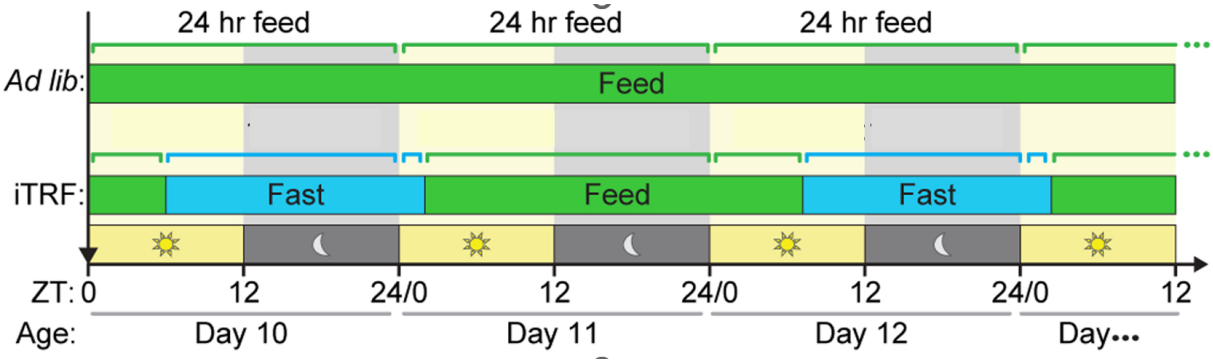


Figure 2: **Feeding schedule for fly cohorts in the Ad lib and iTRF groups (modified from Ulgherait et al., 2021, Nature)**

All flies were raised on *ad lib* conditions until day 10 post-eclosion upon which time flies were placed on various feeding regimes (Figure 2). For consistency, *ad lib* control flies were flipped on to fresh food at the same time as experimental diet flies were transferred to fasting media, and once again when fasting flies were flipped on to regular adult media.

Longevity: flies were placed in each of two vials, per condition (control ad lib, control iTRF, dementia ad lib, dementia iTRF). Flies were transferred to new vials every 3 days to avoid including their offspring in the longevity count. Flies were counted daily, and number of dead flies, number of living flies, and the percentage of surviving flies were recorded. Death was scored at time of flipping, and lifespan compared by log-rank analysis.

Negative Geotaxis or depression like state: Negative geotaxis is defined as the motion in response to the force of gravity (Neckameyer et al. 2016). Flies placed in a vial, were tapped to the bottom, and were given 10 seconds to demonstrate negative geotaxis by migrating upward to a line 2 inches below the vial lid. Number of flies above the demarcated line, at 10 seconds, was recorded (Figure 3). This assay measures both motivation as well as locomotor ability.

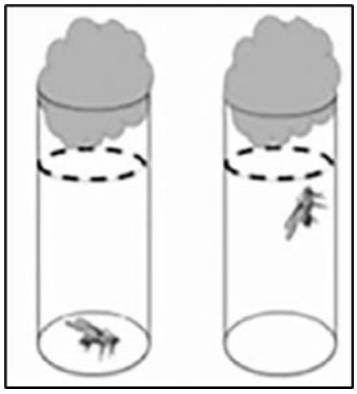


Figure 3: Geotaxis assay to assess lack of motivation and locomotor status.

Learning and Memory

Associative learning and intact memory help organisms, including insects, adapt to their environment which is essential for survival (Rescorla RA, 1988). Fly behavioral assays, involving Pavlovian conditioning tests of memory and learning, are well established (Iijima K 2004; Le Bourg & Buecher (2002). In this study, learned suppression of photopositive tendencies were evaluated in demented and control flies. Phototaxis represents the fly's natural affinity to migrate towards light, but this innate tendency can be suppressed when flies are subjected to an aversive stimulus coupled with light exposure (Le Bourg and Buecher). A choice chamber was created with one end of the chamber covered with opaque black material and the other end open to ambient light. When flies migrate to the lit chamber, they received a noxious stimulus delivered via a buzzing device causing them to leave the lit side. The process is repeated during a 1 minute interval for a total of 5 times and after that the number of flies in the darkened versus light chambers is documented. The proportion flies found in the dark chamber (i.e. the ones who "learned" to avoid potential danger) was defined as the learning index.

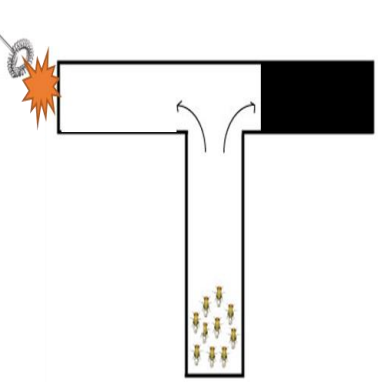


Figure 4: Light-Dark Choice Chamber, where light is coupled with an aversive stimulus.

RESULTS:

Preliminary experimentation found that iTRF (14/10) for 4 days was best tolerated by flies with dementia, although control flies could go longer on this fasting regimen.

Longevity: survival curves were plotted using a Kaplan Meir model (figure 5) and showed that flies without dementia lived longer than demented flies, regardless of which feeling schedule they were subjected to. Within the control and dementia cohorts, the flies subjected to iTRF for 4 days lived longer than flies who fed ad lib, with the effect being more pronounced among the dementia group.

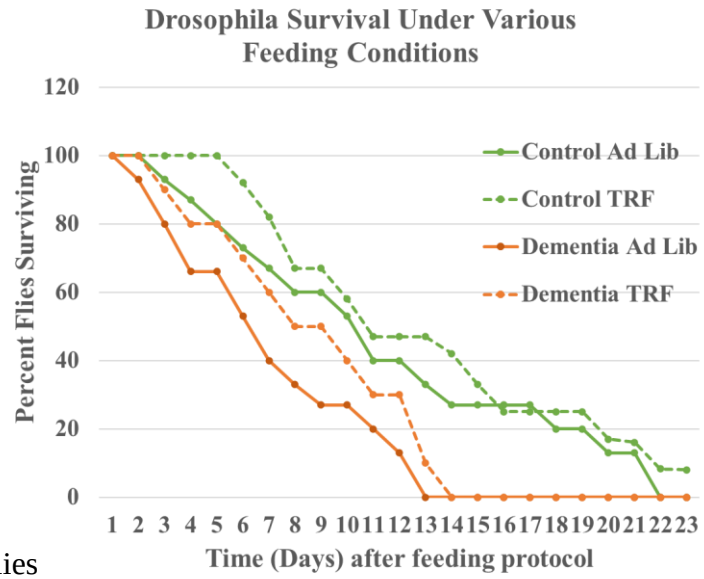


Figure 5: Percent Flies surviving over time

Geotaxis assay: as shown in table 1 and figure 6, Flies with Dementia had impaired climbing ability compared with controls, but within each group, there was no significant difference in locomotor function, among those fed ad lib versus iTRF.

Table 1: Proportion of flies with impaired geotaxis/depression like state, by group

Fly Cohort	n/Total (%)	Odds Ratio: Impaired Geotaxis, TRF vs. ad lib (95% CI)	P Value
CND	3/16 (18.8)	1.0	n/a
CTRF	3/19 (15.8)	1.23 (0.21-7.15)	0.582
Fly Cohort	**n/Total (%)**	**Odds Ratio: Impaired Geotaxis, Dementia TRF vs. ad lib (95% CI)**	**P Value**
DND	8/24 (33.3)	1.0	n/a
DTRF	4/12 (33.3)	1.0 (0.23 – 4.35)	0.64

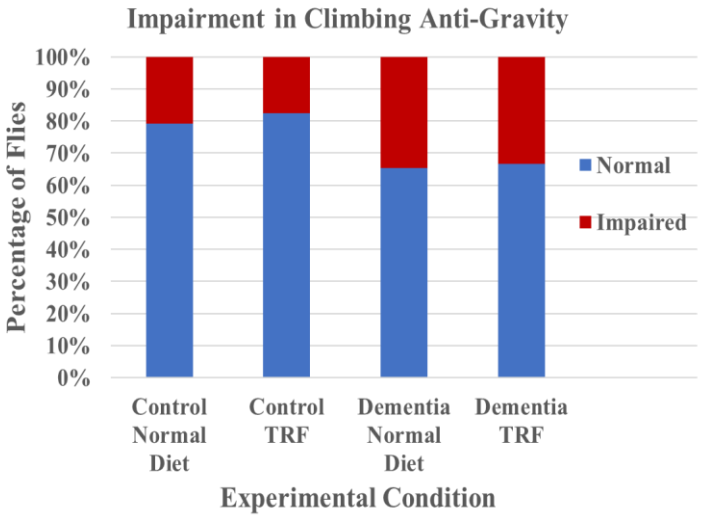
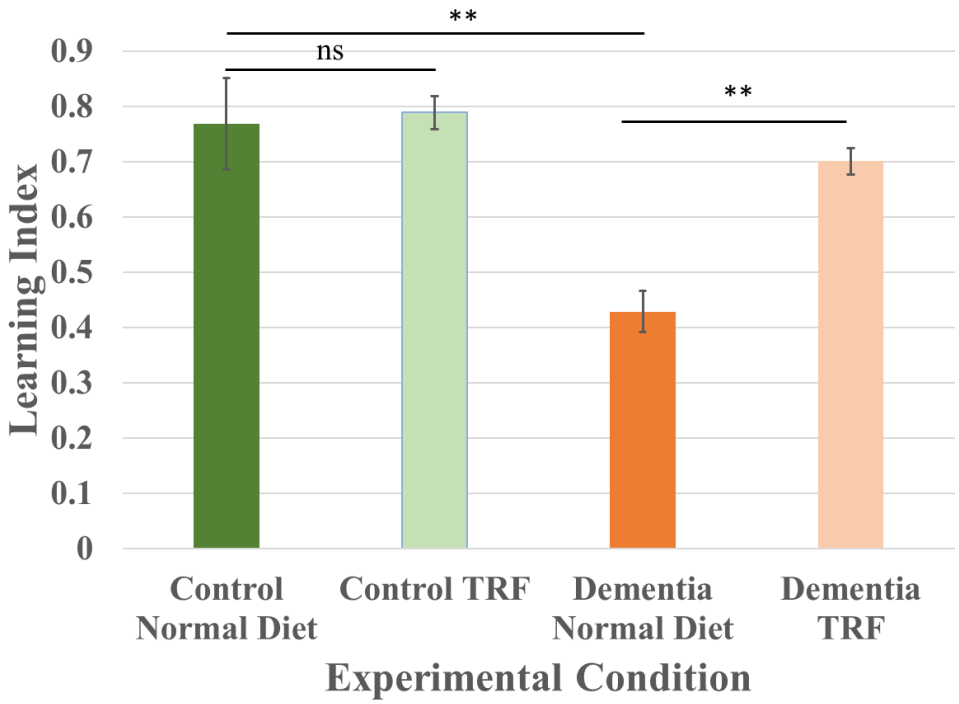


Figure 6: Indicated above is the percentage of flies with impaired geotaxis for the various groups.

Demented flies had 33% impairment in climbing compared to only 19 percent among controls. However, within the controls there was no difference between the ad lib and iTRF group (p-value = 0.582) and there was no difference between the two diet s for the demented group either (p=value = 0.64).

Learning and Memory: After receiving an aversive stimulus, the number of flies who "learned" to avoid light were recorded over 3 trials. Learning index was defined as the percentage of flies that successfully suppressed phototaxis (or the innate desire to migrate towards light) and migrated to the unlit side of the chamber. As shown in figure 7, flies with dementia demonstrated significant impairment in short-term memory and learning compared to the control flies (p-value = 0.03). Notably, iTRF did not improve short-term memory among control flies (p-value = 0.55) but greatly enhanced memory and learning in the dementia group (p-value = 0.04).



ns – not significant ** - p-value <0.05

Figure 7: Short-term Memory Evaluation by Aversive Phototaxis Suppression Assay. Learning Index calculated based on percentage of flies who "learned" to avoid light

DISCUSSION/ CONCLUSIONS:

The purpose of this study was to utilize *Drosophila melanogaster* as a model organism and study whether metabolic manipulation using time restricted feeding could improve mortality, locomotion, and memory among demented and control flies. Flies with the V337 MAP Tau mutation demonstrated impairments consistent with dementia as previously described (Spina et al., 2017) – they have shorter life spans, impaired climbing and motivation, and deficits in learning and memory, when compared with control flies. Intermittent time restricted feeding or iTRF enhanced lifespans of both control and demented flies but did not improve locomotor abilities in either group. Flies with dementia, but not the controls, showed improved learning and memory after iTRF.

iTRF and Mortality

The results of this study are consistent with prior literature showing dementia is a reliable predictor of human mortality (Rizzuto et al., 2012). Caloric restriction via intermittent fasting in humans (Dowis and Banga, 2021) and time restricted feeding in flies (Ulgherait et al., 2021) can prolong lifespan. In this project, iTRF decreased mortality among flies with dementia and showed that although they may be more physically vulnerable, they can also benefit from ketosis.

iTRF and Climbing Behavior

While demented flies had impaired climbing compared with control flies, iTRF did not improve locomotion in either group. Negative geotaxis in flies is the innate motivation to climb vertically when startled; impairment in this response has been observed in flies with lower serotonin and octopamine levels (Meichtry et al., 2020), which are associated with depression in flies. Intact geotaxis also requires good locomotor ability and negative geotaxis has been shown to be impaired among flies with neurodegenerative processes such as Alzheimer and Parkinson disease, as well as flies with brain injury or Chronic Traumatic Encephalopathy (Lateef et al., 2019). The neurodegeneration brought on by hyperphosphorylated tau causes parkinsonism, in addition to dementia, which may explain why iTRF was not successful in overcoming these deficits in this model of dementia.

iTRF and Memory

Flies with dementia, but not the controls, showed improved learning and memory after iTRF possibly because it is easier to discern enhanced cognition among flies who are significantly impaired to begin with. The three ketone bodies produced during fasting are acetoacetate, β-hydroxybutyrate and acetone and they are the major energy source (75%) for the brain. The proposed neuroprotective effects of ketone bodies include decreasing oxidative stress, neuroinflammation, mitochondrial impairment, hypometabolism and BBB disruption (Lorenzo, 2018). Especially relevant to the model of dementia used in this study, impaired glucose metabolism leads to a decrease in tau-O-GlyNAcylation that causes more hyperphosphorylation of tau (Lorenzo, 2018). By switching the fly metabolism to ketosis, it is possible that hyperphosphorylation of tau was diminished leading to less neuropathology and this improved cognitive function.

Findings from this study have tremendous implications for the treatment of dementia, a highly prevalent and devastating neurologic diagnosis. Intermittent fasting is a non-pharmacologic intervention that can be easily prescribed and perhaps the exact feeding schedule can be adjusted to meet the needs of the individual patient. It is also less restrictive than the ketogenic diet which necessitates the almost complete elimination of carbohydrates and can lead of adverse effects such as liver and kidney disease and vitamin deficiencies (Batch et al., 2020).

This study was limited by space and budget considerations and more extensive replicates, with different feeding schedules were not possible. Additionally, due lack of access to specialized equipment, ketosis could not be biochemically assessed in the flies.

iTRF may be a promising new intervention for dementia. Future studies should evaluate the associated metabolic pathways, as well as the changes in brain pathology, that accompany improvement in clinical symptoms, both in flies and in larger mammalian models. Ultimately such non-pharmacologic interventions may transform how we treat a variety of human neurodegenerative diseases.

LITERATURE CITED

Peer-reviewed

Batch, J. T., Lamsal, S. P., Adkins, M., Sultan, S., & Ramirez, M. N. (2020). Advantages and Disadvantages of the Ketogenic Diet: A Review Article. *Cureus*, 12(8), e9639. https://doi.org/10.7759/cureus.9639

Bonner JM, Boulianne GL: Drosophila as a model to study age-related neurodegenerative disorders: Alzheimer's disease. *Exp Gerontol*. 2011, 46: 335-339. 10.1016/j.exger.2010.08.004.

Clifford R Jack, Jr, Heather J Wiste, Hugo Botha, Stephen D Weigand, Terry M Therneau, David S Knopman, Jonathan Graff-Radford, David T Jones, Tanis J Ferman, Bradley F Boeve, Kejal Kantarci, Val J Lowe, Prashanthi Vemuri, Michelle M Mielke, Julie A Fields, Mary M Machulda, Christopher G Schwarz, Matthew L Senjem, Jeffrey L Gunter, Ronald C Petersen, The bivariate distribution of amyloid-β and tau: relationship with established neurocognitive clinical syndromes, *Brain*, Volume 142, Issue 10, October 2019, Pages 3230–3242, https://doi.org/10.1093/brain/awz26

Daneman R, Prat A. The blood-brain barrier. Cold Spring Harb Perspect Biol. 2015 Jan 5;7(1):a020412. doi: 10.1101/cshperspect.a020412. PMID: 25561720;

Dowis, K., & Banga, S. (2021). The Potential Health Benefits of the Ketogenic Diet: A Narrative Review. *Nutrients*, *13*(5), 1654. https://doi.org/10.3390/nu13051654

Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, et al: Global prevalence of dementia: a Delphi consensus study. *Lancet*. 2005, 366: 2112-2117

Gale, S. A., Acar, D., & Daffner, K. R. (2018). Dementia. *The American journal of medicine*, 131(10), 1161–1169. https://doi.org/10.1016/j.amjmed.2018.01.022

Hertz, L., Chen, Y., & Waagepetersen, H. S. (2015). Effects of ketone bodies in Alzheimer's disease in relation to neural hypometabolism, β-amyloid toxicity, and astrocyte function. *Journal of neurochemistry*, *134*(1), 7–20. https://doi.org/10.1111/jnc.13107

Iijima K. Dissecting the pathological effects of human Aβ40 and Aβ42 in Drosophila: A potential model for Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 2004;**101**:6623–6628.

Iqbal K, Liu F, Gong CX, Grundke-Iqbal I: Tau in Alzheimer disease and related tauopathies. *Curr Alzheimer Res.* 2010, 7: 656-664. 10.2174/156720510793611592.

Lateef, S., Holman, A., Carpenter, J., & James, J. (2019). Can Therapeutic Hypothermia Diminish the Impact of Traumatic Brain Injury in Drosophila melanogaster? *Journal of experimental neuroscience*, 13, 1179069518824852. https://doi.org/10.1177/1179069518824852

Le Bourg, E., & Buecher, C. (2002). Learned suppression of photopositive tendencies in Drosophila melanogaster. *Animal learning & behavior, 30*(4), 330–341.

Lessing D, Bonini NM. Maintaining the brain: insight into human neurodegeneration from Drosophila melanogaster mutants. *Nat Rev Genet*. 2009 Jun;10(6):359-70. doi: 10.1038/nrg2563.

Lorenzo, P. A. (2018). Alzheimers Disease as a metabolic pathology: new approaches [Degree Project, Universitat de Barcelona]. http://diposit.ub.edu/dspace/bitstream/2445/125012/1/TFG_diposit_Alvarez%20Lorenzo.pdf

McGuire SE, Deshazer M, Davis RL. Thirty years of olfactory learning and memory research in Drosophila melanogaster. *Prog Neurobiol*. 2005;76:328–347. doi: 10.1016/j.pneurobio.2005.09.003.

Nasaruddin ML, Syed Abd Halim SA, Kamaruzzaman MA. Studying the Relationship of Intermittent Fasting and β-Amyloid in Animal Model of Alzheimer's Disease: A Scoping Review. *Nutrients*. 2020 Oct 21;12(10):3215. doi: 10.3390/nu12103215.

Poudel P, Park S. Recent Advances in the Treatment of Alzheimer's Disease Using Nanoparticle-Based Drug Delivery Systems. *Pharmaceutics*. 2022 Apr 11;14(4):835. doi: 10.3390/pharmaceutics14040835

Prüßing K, Voigt A, Schulz JB. Drosophila melanogaster as a model organism for Alzheimer's disease. *Mol Neurodegener*. 2013 Nov 22;8:35. doi: 10.1186/1750-1326-8-35. PMID: 24267573;

Pugazhenthi S, Qin L, Reddy PH. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta Mol Basis Dis*. 2017 May;1863(5):1037-1045. doi: 10.1016/j.bbadis.2016.04.017. Epub 2016 May 6.

Ratnavalli, E., Brayne, C., Dawson, K., & Hodges, J. R. (2002). The prevalence of frontotemporal dementia. *Neurology*, *58*(11), 1615–1621. https://doi.org/10.1212/wnl.58.11.1615

Rescorla RA. Behavioral studies of Pavlovian conditioning. *Annu Rev Neurosci*. 1988;11: 329–352.

Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L: Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science*. 2007, 316: 750-754. 10.1126/science.1141736.

Rizzuto, D., Bellocco, R., Kivipelto, M., Clerici, F., Wimo, A., & Fratiglioni, L. (2012). Dementia after age 75: survival in different severity stages and years of life lost. *Current Alzheimer research*, 9(7), 795–800. https://doi.org/10.2174/156720512802455421

Spillantini MG, Crowther RA, Goedert M. Comparison of the neurofibrillary pathology in Alzheimer's disease and familial presenile dementia with tangles. *Acta Neuropathol* 1996; 92:42–48

Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci* USA 1998;95:7737–7741.

Spina S, Schonhaut DR, Boeve BF, Seeley WW, Ossenkoppele R, O'Neil JP, Lazaris A, Rosen HJ, Boxer AL, Perry DC, Miller BL, Dickson DW, Parisi JE, Jagust WJ, Murray ME, Rabinovici GD. Frontotemporal dementia with the V337M *MAPT* mutation: Tau-PET and pathology correlations. *Neurology.* 2017 Feb 21;88(8):758-766. doi: 10.1212/WNL.0000000000003636. Epub 2017 Jan 27.

Sumi SM, Bird TD, Nochlin D, Raskind MA. Familial presenile dementia with psychosis associated with cortical neurofibrillary tangles and degeneration of the amygdala. *Neurology* 1992; 42:120–127

Taylor MK, Sullivan DK, Swerdlow RH, Vidoni ED, Morris JK, Mahnken JD, et al. A high-glycemic diet is associated with cerebral amyloid burden in cognitively normal older adults. *Am J Clin Nutr.* (2017) 106:1463– 70. doi: 10.3945/ajcn.117.162263

Ulgherait M, Midoun AM, Park SJ, Gatto JA, Tener SJ, Siewert J, Klickstein N, Canman JC, Ja WW, Shirasu-Hiza M. Circadian autophagy drives iTRF-mediated longevity. *Nature.* 2021 Oct;598(7880):353-358. doi: 10.1038/s41586-021-03934-0. Epub 2021 Sep 29

Zhang, Y., Wu, KM., Yang, L. *et al.* Tauopathies: new perspectives and challenges. *Mol Neurodegeneration* **17**, 28 (2022). https://doi.org/10.1186/s13024-022-00533-z

Non-Peer Reviewed

Dementia. **https://www.who.int/health-topics/dementia#tab=tab_1. Accessed 26 Dec 2021.**