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Elucidating the Effects of Glucose Toxicity on Tauopathy and Aging

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Elucidating the Effects of Glucose Toxicity on Tauopathy and Aging

By

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A culminating thesis submitted to the faculty of Dominican University of California in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences

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Abstract

Diabetes patients are at higher risk of contracting an age-related neurodegenerative disease such as Alzheimer's disease (AD). However, the mechanisms which link these diseases are poorly understood. We hypothesize that glucose and elevated levels of the glycolysis by product advanced glycation end-products (AGEs), may be involved. AGEs accumulate with age and are elevated in both diabetic and AD patients. Diabetes is a metabolic disorder for which consumption of sugar-rich diets is a major risk factor and is central to etiology in the vast majority of cases.

We show that transgenic *C. elegans* expressing wild type (WT) human tau fed a diet supplemented with glucose drastically reduces lifespan, increases feeding behavior, reduced motility, and results in the accumulation of AGEs. Interestingly, *C. elegans* expressing A152T mutant human tau had improved lifespan and motility when given a high glucose diet, indicating this tau mutation interacts with metabolism in a drastically different manner. Tau expressing animals (WT and A152T) fed the AGE, methylglyoxal-derived methyl-glyoxal-hydroimidazolone (MGH1), have shortened lifespans, increased feeding, and reduced motility. Tau expression appears to have general impacts on overall gene expression, as human tau expressing worms (WT and A152T) exhibit disrupted fat metabolism and glyoxalase expression. Therefore, our data supports the hypothesis that glucose and AGEs may act as a mechanistic link between Alzheimer's disease, other tauopathies, and diabetes, and that therapies aimed at inhibiting AGE formation may prove beneficial in treating these diseases.

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Thank you to Pankaj Kapahi, PhD for hosting me in his lab and allowing me to do this research. Special thanks to Aimee Kao, MD, PhD for generating and providing our lab with *C. elegans* strains used in this research. The Kapahi lab members and many others at the Buck Institute deserve acknowledgement for their encouragement, mentoring, and friendship over the course of this work. Specifically I would like to thank Kristeen Pareja-Navarro, PhD for her mentorship and troubleshooting help, Harshani Peiris, PhD, Muniesh Shanmugam, PhD, and Durai Sellegounder, PhD for their guidance in *C. elegans* models, and Manish Chamoli, PhD and Cyrene Arputhasamy for their help over the course of this project. Last, but most importantly, I thank my family for their unending support.

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Introduction

The number of people in the United States affected by Alzheimer's disease (AD) or other dementias is quickly escalating, and currently an estimated 5.7 million Americans of all ages are living with Alzheimer's dementia[1]. In 2017, caregivers of people with Alzheimer's or other dementias provided an estimated 18.4 billion hours of informal (unpaid) assistance, valued at $232.1 billion[1]. This illustrates how costly dementia related diseases are for society and how important research on Alzheimer's and other dementias is. A major risk factor for AD is age, but there are other risk factors including obesity and diabetes, which significantly and independently increase the risk for AD[2].

Advancements in science and technology have improved the understanding risk factors associated with AD, but the correlation between diabetes and AD is still not well understood. Increasingly, evidence such as insulin resistance and disrupted energy metabolism in AD brains suggests that AD is actually a metabolic disease at its core[3–5]. The commonality of metabolic disorder in both diabetes and AD may be what links the two diseases and merits further research.

A distinguishing feature of diabetes is high blood sugar (hyperglycemia) due to insulin resistance, and may be important for the connection to AD. The increase in sugar consumption has long been linked to the rise in many non-communicable diseases [6]. Chronic hyperglycemia results in elevated levels of highly reactive α-dicarbonyl compounds (α-DCs) such as methylglyoxal (MGO) which eventually lead to an accumulation of advanced glycation endproducts[7–9]. MGO is formed spontaneously from glyceraldehyde-3-phosphate and dihydroxyacetone phosphate during glycolysis[10,11]. α-DCs react indiscriminately with proteins, lipids, and DNA to yield advanced glycation end-products (AGEs)[12–14] (**Fig. 1**). Interestingly, the accumulation of AGEs have also been associated with aging and age-related diseases including

AD and diabetes [8,15–17]. The formation of AGEs causes irreversible damage to the macromolecule that is modified, changing the structural and functional integrity[8]. AGEs promote proteotoxicity, cellular stress, and inflammation, through binding cell surface receptors, which may induce deleterious signaling/block beneficial signaling, or cross-linking with proteins[7,18,19]. AGEs can also be consumed through diet, particularly in baked or seared foods where the Maillard reaction takes place and forms AGEs.

Detoxification of α-DCs is primarily attributed to glutathione-dependent glyoxalase, GLO1[11], and co-factor-independent glyoxalase, DJ1[20], both evolutionarily conserved. AGEs accumulate during aging and there is evidence that AGEs have a role in the pathogenesis of multiple age-related diseases, including diabetic pathologies and neurodegenerative diseases[7,15,16,21–24].

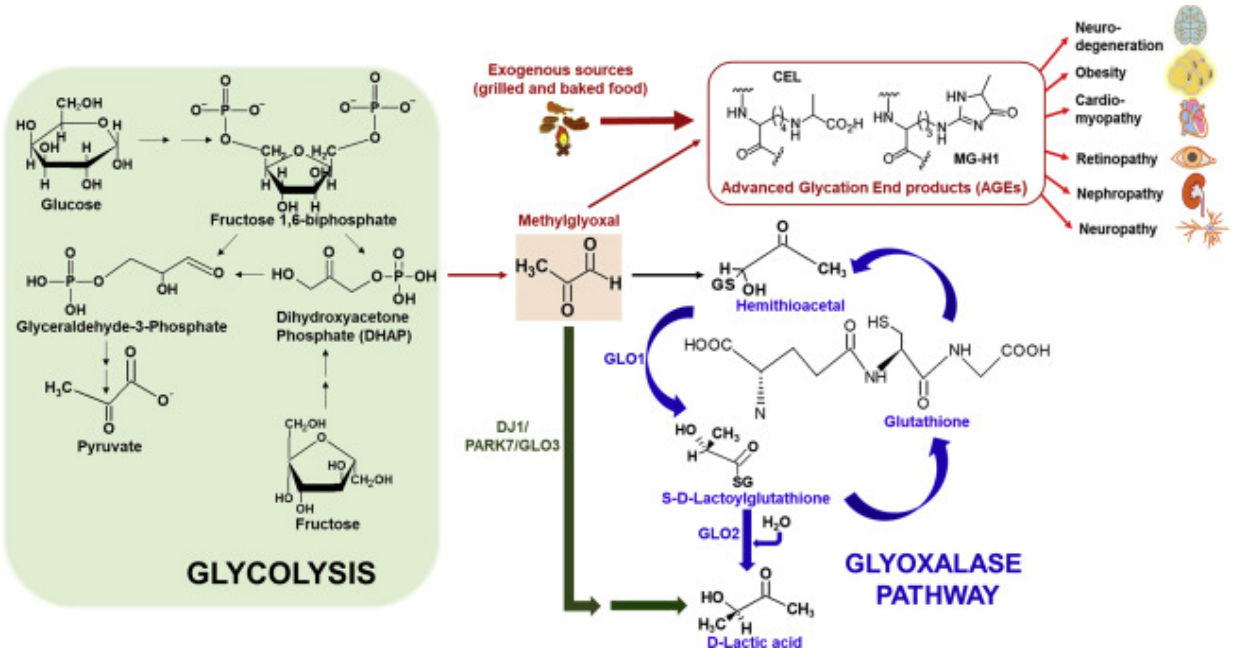

Figure 1: Formation of AGEs and the Glyoxalase pathway.

MGO is an unavoidable byproduct of glycolysis which irreversibly interacts with biomolecules to from AGEs. It is also important to note that grilled and baked food is also a major source of AGEs. The glyoxalase pathway detoxifies MGO through the enzymes glyoxalase I (GLO1) and glyoxalase II (GLO2), eventually converting MGO to lactic acid. Alternatively, MGO can be converted to lactic acid in a single step through DJ1/PARK7/GLO3.

Hallmark pathologies of AD are the accumulation of β-amyloid plaques and hyperphosphorylated tau aggregates[25]. Tau proteins normally associate with microtubules and are essential for stabilizing neuronal structure[26,27]. However, dysfunctional tau can become toxic, affecting neuronal health, and is a major characteristic of AD[26]. Pathologies associated with tau deposits are collectively called tauopathies.

One of the leading theories for tau dysfunction in AD and other tauopathies is hyperphosphorylation of tau[26]. Phosphorylation is a type of post translational modification (PTM) usually affecting serine, threonine, and tyrosine residues, in which a phosphate group is added to the target residues by protein kinases[28]. PTMs are employed by organisms to regulate a multitude of cell activities[28,29]. In the case of tau, increased phosphorylation can interfere with microtubule binding, creating a toxic loss of function, and increase tau-tau interactions (neurofibrillary tangles) resulting in a toxic gain of function[26,29]. Other post-translational modifications may contribute to tau toxicity, including glycation[30]. Endogenous tau has been shown to have up to 63 unique post-translational modifications[31]. It's complex PTMs indicates that tau is heavily regulated by diverse mechanisms[31].

Recent evidence suggests that AGEs can induce tau phosphorylation through the RAGE-AGE axis[32]. The receptor for advanced glycation end products (RAGE) is a receptor in the immunoglobulin superfamily[33]. It is believed that AGEs binding RAGE can cause complications in multiple diseases such as diabetes, neurodegeneration, and inflammation.

In order to further understand the effect of AGEs *in vivo* our lab has previously studied the *C. elegans* mutant *glod-4* which is deficient in the α-DC detoxifying glyoxalase *glod-4* (orthologue of human *GLO1*). This deficiency causes an accumulation of AGEs and the animals

have a reduced lifespan, neuronal damage, and hypersensitivity to touch[34]. We have since discovered that inhibition of the enzyme tyrosine decarboxylase (*tdc-1*), responsible for the biosynthesis of the neurotransmitter tyramine from the amino acid tyrosine, rescues the lifespan reduction in *glod-4* mutants (**Fig. 2**). The role of *tdc-1* in the glyoxalase pathway merits further study.

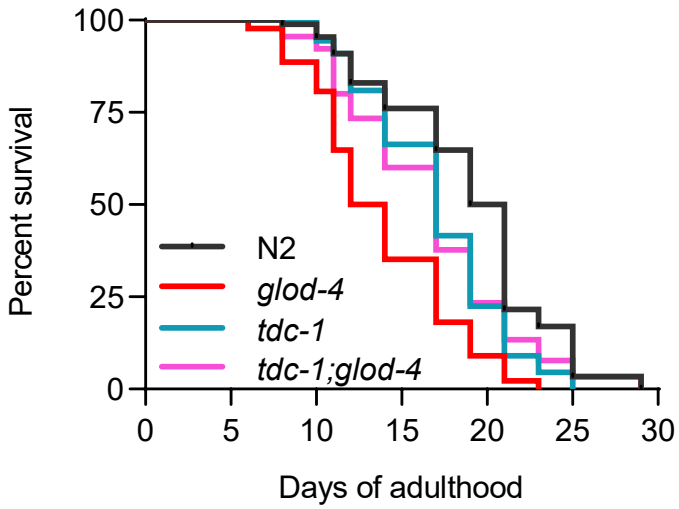

Figure 2: Lack of tyramine suppresses the adverse effect of glod-4 mutants.

A) Survival curves of N2, *glod-4*, *tdc-1* and *tdc-1; glod-4* double mutants. Log rank (Mantel-Cox) test performed for significance.

In the current study, we were interested to explore how tau is affected by hyperglycemia, AGEs, and *tdc-1*. To study this we used C. elegans tauopathy models created by Aimee Kao's lab at UCSF[35]. The first model, CF3810, expresses human wild-type (wt) tau (1N4R isoform) pan-neuronally under the *aex-3* promoter. The second model, CF3827, expresses human A152T mutant tau (1N4R isoform) pan-neuronally under the *aex-3* promoter. 1N4R is the most abundant isoform of tau in the adult human brain[36,37]. A152T is a rare mutation of the tau protein which has been shown to confer an additional phosphorylation site[35]. The A152T mutation has been

indicated in increasing the risk for FTLD, AD, and Parkinson's disease[35,38]. It was of interest to us how this mutation affects metabolism, glyoxalase activity, and lifespan in a hyperglycemic condition, and what role AGEs may play in its pathology.

Experimental Design & Methods

C. elegans Strains and Maintenance

The N2 Bristol strain (obtained from Caenorhabditis Genetics Center (CGC)) was used as the wild type control strain. Transgenic strains CF3810 *N2E; muIs216 [aex-3p::huMAPT 4RIN + myo-3p::rfp]*, CF3827 *N2E; muIs217 [aex-3p:: huMAPT 4RIN A152T + myo-3p::rfp]*. were generously gifted by Dr. Aimee Kao, MD, PhD, University of California, San Francisco. All strains were maintained at 16°C or 20°C on Nematode Growth Media (NGM) following general worm handling and maintenance practice as previously described[39]. 2% glucose NGM was made for experiments by substituting water in the NGM recipe with a solution of 40% glucose in water, or by adding a solution of 40% glucose in water on top of the plate. Nystatin (10μg/mL) and streptomycin (100 μg/mL) were used in NGM to prevent contaminations. NGM was seeded with *Escherichia coli* OP50-1 as a food source for C. elegans. In all assays care was taken to maintain healthy worms and prevent starvation.

C. elegans Lifespan Studies

Lifespans of synchronized worms were performed as previously described with minor changes[35]. Briefly, 100 synchronized young adult worms were transferred to regular NGM plates or NGM plates supplemented with 2% glucose. Plates were seeded with *Escherichia coli* OP50-1. Worms were scored every day for survival by gently prodding the worms head with a platinum wire cover in bacteria. Worms that failed to respond to prodding were scored as dead. Animals that crawled off the plate or bagged due to internal hatching were censored from the study. Worms were transferred to new plates every day for the first week until worms stopped laying eggs, then animals were transferred every 2-3 days.

Thrashing Assay

Thrashing assay was performed as previously described[35]. Briefly, animals were grown on control media, 2% glucose media, 150 µM Arginine control media, or 150 µM MGH1 media until Day 4 of adulthood. Worms were placed in a drop of M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 mL 1M $MgSO_4$, in 1000 mL DI H_2O) and allowed to equilibrate for 1 minute. Then the number of thrashes were counted over a 1-minute time period. A "thrash" was defined as the movement of the worm head from one side to the other side, and back to the original side.

Activity Assay

Synchronized worms were grown on control media, 2% glucose media, 150 µM Arginine control media, or 150 µM MGH1 media until day 1 young adult stage. Animals were collected with M9 buffer and washed three times with M9 buffer to remove OP-50-1 food. Worm solutions were concentrated to 300 worms per 100 µL. 100 µL of each worm solution was aliquoted to a well in a 96 well plate, in triplicate. Average activity units were measured using the WMicrotracker One™ (PhylumTech) system over 30 minutes.

Pumping Assay

Pumping assays were performed as previously described[35]. Briefly, animals were grown on either normal control media, 2% glucose media, 150 µM Arginine control media, or 150 µM MGH1 media until young adult day 1. Pumping rates were analyzed as the number of contractions of the terminal bulb over 30 seconds.

Triglyceride Assay

Triglyceride content in day 4 adult worms was measured after growing on regular media or 2% glucose media. Assay utilized the Stanbio LiquiColor Triglycerides kit following manufacturer's instruction. Normalized to protein content.

RNA isolation and RT-qPCR

Total RNA was isolated from synchronized Day 4 adult worms grown on either normal control media, 2% glucose media, 150 µM Arginine control media, or 150 µM MGH1 media. Animals were collected from plates with M9 buffer and washed three times using M9 buffer to remove OP50-1 food. Worm pellets were flash frozen in liquid nitrogen. Frozen worm pellets were thawed on ice, resuspended in lysis buffer (Zymo Research, cat.# R1060-1-50) and incubated at 55°C for 50 mins. RNA was extracted and purified using Zymo Research Quick-RNA MiniPrep Kit following manufacturer protocol (Zymo Research, cat# R1055). Reverse transcription (500 ng RNA per sample) was performed using BioRad iScript Reverse Transcription Supermix for RT-qPCR (BioRad, Cat. #1708841) to create cDNA for qPCR. qPCR was performed using Bioline SensiFAST SYBR No-ROX Kit (Bioline, Cat. No. BIO-98020) in a Roche LightCycler480 II System. Briefly, qPCR was performed using 10 µL reactions with 1 µM primers (**Table 1**), 1X SensiFAST SYBR, and 1:3 dilution of cDNA. Double delta Ct analysis was performed normalizing to the *C. elegans* housekeeping gene *act*-5. Data is displayed relative to N2 control.

Table 1: PCR Primers

Primer	**Sequence**
fat-6 forward primer	5'-TCGGACTCTACCAGCTCATC-3'
fat-6 reverse primer	5'-TGATTTGTGGGACCAGAGAC-3'
nhr-49 forward primer	5'-ACGGGTGTAAGGGATTCTTC-3'
nhr-49 reverse primer	5'-TGAAAGCGACAATAACGACA-3'
lbp-5 forward primer	5'-GAATTCGACGAGACAACACC-3'
lbp-5 reverse primer	5'-TTCTCCTTCAAACCAACGAG-3'
mdt-15 forward primer	5'-GGTGGAAATCCGTACAATCA-3'
mdt-15 reverse primer	5'-TTCCAGGATATCCATGTGGT-3'
act-5 forward primer	5'-TGCTTTCCTTGTACGCTTCC-3'
act-5 reverse primer	5'-AAGATCGAGACGTTGGATGG-3'
glod-4 forward primer	5'-GGATATGGCTCAGAGGATGA-3'
glod-4 reverse primer	5'-GAATCGATGACGATTGCTCT-3'
djr1.1 forward primer	5'-CATGGAGTCAAGGCAGAACT-3'
djr1.1 reverse primer	5'-TCCACGAGAGGTGATGATTT-3'
djr1.2 forward primer	5'-GCCGAAGAAATCGAGGTTAT-3'
djr1.2 reverse primer	5'-GAGCTACGTCCGGAACAATA-3'
tpi forward primer	5'-TTTTCTGCATTGGGGAGAAG-3'
tpi reverse primer	5'-GGTTCGTAGGCGATCACAAT-3'

Cell Culture

Transgenic SH-SY5Y neuroblastoma cells expressing human tau or P301L mutant human tau were obtained courtesy of Dr. Jürgen Götz[40].

SH-SY5Y cells were grown in DMEM/F12 1:1 Modified medium (HyClone, Cat. No. SH30261.01) with 10% fetal bovine serum (FBS, Millipore Sigma, SKU: F0926) and 1X

Penicillin Streptomycin Solution (Coring, Ref: 30-002-CI). Cells were incubated at 37°C in a humidified air incubator containing 10% CO_2 and 3% O_2.

Protein Extraction

For *C. elegans* experiments, immunoblotting was performed as previously described[35]. Briefly, day 1 adult animals and day 4 adult animals, grown on either normal control media, 2% glucose media, 150 μM Arginine control media, or 150 μM MGH1 media, were collected from plates with ice cold M9 and washed twice to remove OP50-1 food. M9 was removed and the worm pellet resuspended 1:1 with freshly made ice cold RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% SDS, 0.5% SDO, 1% NP-40, 1 mM PMSF, cOmplete protease inhibitor (Roche #04693124001) and PhosSTOP phosphatase inhibitor (Roche #4906837001).

For cell culture experiments, SH-SY5Y cells were grown in T25 cell culture. Okadoic acid control flasks were treated with 75 nM okadoic acid for 1.5 hours before being harvested. Cells were harvested by washing the flask once with phosphate buffered saline (PBS, Sigma, P3813) then using trypsin (Coring, 0.25% Trypsin, 2.21 mM EDTA, 1X[-] sodium bicarbonate, REF: 25-053-CI) to detach cells from flasks. Trypsin was then inactivated with an equal volume of fresh growth media. Cells were spun at 4000 rpm for 5 mins. Cell pellets were washed with ice cold PBS and the cell pellet allowed to dry, then flash frozen in liquid nitrogen and stored at -80°C. On ice, frozen cell pellets were resuspended in Cell Lysis Buffer (CLB, 150 mM sodium chloride, 1.0% NP-40, 50 mM Tris pH 8.0) with protease inhibitor (Roche, cOmplete, Mini, EDTA-free, protease inhibitor cocktail, SKU: 11836170001) and phosphatase inhibitor (Roche, PhosSTOP, SKU: 4906845001). Suspended cells were sonicated for 5 cycles (30 seconds on, 30 seconds off) in a Diagenode BIORUPTOR300. Cell lysate was centrifuged in 4°C for 20 mins at 15000 rpm and the supernatant was collected and stored at -80°C.

Immunoblotting

Protein extract was quantified for protein concentration using the bicinchoninic acid assay (BCA assay, BioVision, BCA Protein Assay Kit II, K813-2500). 30-50 µg total protein was resolved on NuPAGE 4-12% Bis-Tris Gels (Thermo Fisher Scientific, REF: NP0336BOX) and transferred to polyvinylidene difluoride (PVDF) membrane using an iBlot™ Gel Transfer Device (Thermo Fisher Scientific) and iBlot Gel Transfer Stacks PVDF, Mini (Thermo Fisher Scientific, REF: IB401002). Phosphorylated tau levels were immunoblotted with Phospho-Tau (Ser202, Thr205) Monoclonal Antibody AT8 (Thermo Fisher Scientific, Catalog # MN1020), tau monoclonal antibody HT7 (ThermoFisher Cat# MN1000). MG-H1 levels were probed with mouse anti-MG-H1 antibody 1H7G5 (1:1000) (Novus, Cat# NBP2-62810) Loading control was immunoblotted with β-Actin Antibody (1:4000) (Cell Signaling, #4967) or alpha-tubulin (1:4000) (DSHB, #12G10). Protein ladder used for size reference was ProSignal Full-Range Prestained Protein Ladder (Genesee Scientific Cat # 83-650)

Results

Glucose shortens lifespan of control strains but increases lifespan of A152T-Tau *C. elegans* mutants

It has been shown previously that glucose significantly shortens the lifespan of C. elegans[41,42]. Furthermore, hyperglycemia has been implicated in the pathogenesis of AD[43,44]. For this study, we wanted to determine if tau protein might affect the lifespan effect of glucose (**Fig. 3**). Synchronized L1 animals were grown on either regular Nematode Growth Media (NGM) or NGM supplemented with 2% glucose. Plates were seeded with *Escherichia coli* OP50-1 food. Wild-type N2 worms and transgenic worms expressing human wild-type tau (CF3810) live significantly shorter lifespans in high glucose conditions. Interestingly, the very short lifespan of A152T-Tau animals was rescued when these animals were treated with glucose (**Fig. 3 B**).

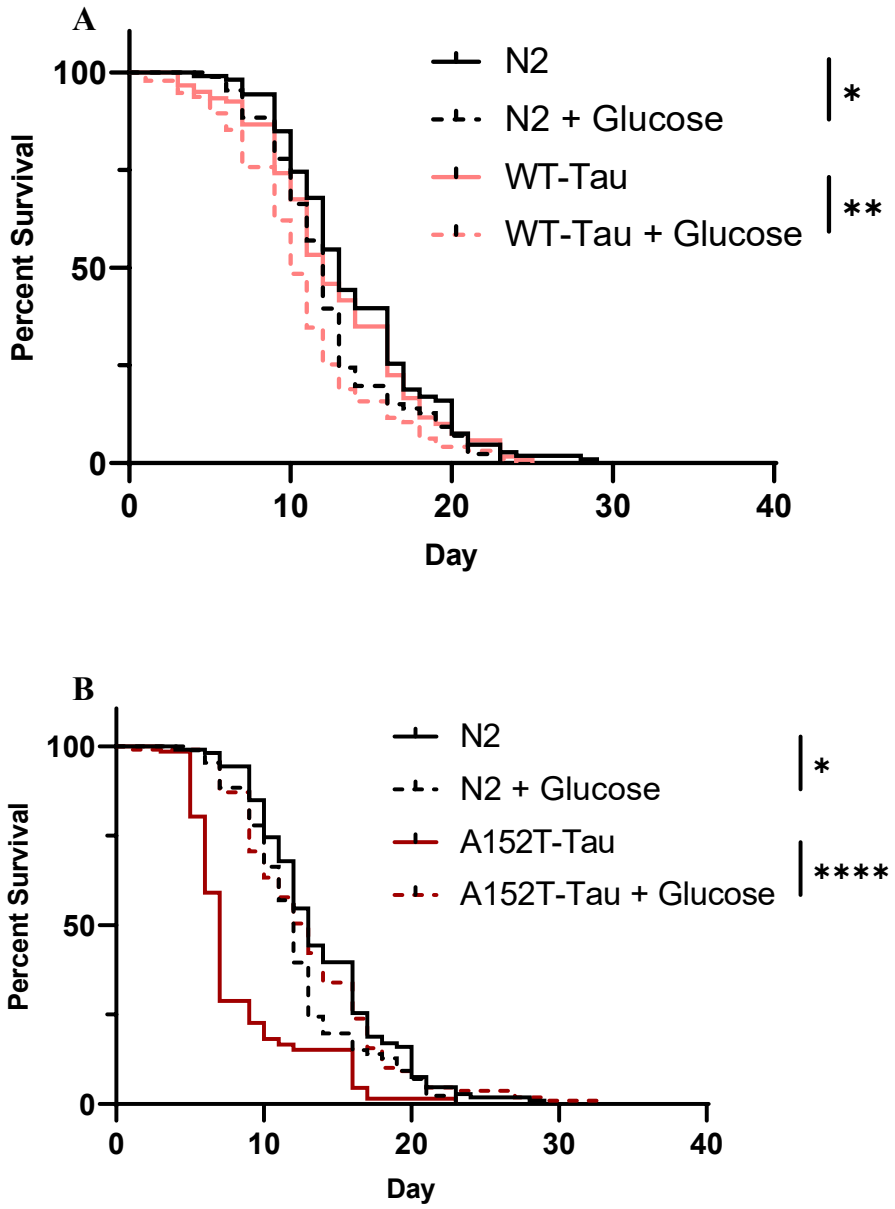

Figure 3: Increase in dietary glucose shortens *C. elegans* lifespan, but rescues A152T mutant tau lifespan.

(**A**) Lifespan survival curve for control non-transgenic N2 animals and transgenic WT-Tau expressing animals grown on either regular media or 2% glucose media. (**B**) Lifespan survival curve for control non-transgenic N2 animals and transgenic A152T-Tau expressing animals grown on either regular media or 2% glucose media. (**A-B**) Log-rank (Mantel-Cox) test was performed to determine statistical significance, P=0.0214 (*) N2 compared to N2 + 2% glucose, P=0.0027(**) WT-Tau compared to WT-Tau + 2% glucose, P<0.0001 (****) A152T-Tau compared to A152T-Tau + 2% glucose.

Glucose increases feeding behavior and reduces motility in *C. elegans*

After observing the negative effects of glucose on N2 and WT-Tau animals but a positive effect on A152T-Tau animals, we wanted to better understand how this differential effect is occurring. To understand this, we performed a panel of experiments that explored C. elegans feeding, mobility/activity, and the ability to accumulate or reduce body fat. The pumping assay measures how frequently the terminal bulb in the mouth of the animal is contracting. This is a good indication of the animals feeding behavior. Worms grown on 2% glucose media had significantly higher pumping rates than those on regular NGM, indicating glucose had triggered an increase in feeding behavior (**Fig. 4A**). The thrashing (body bending) assay is a good measurement of the worm's motility, where low thrashing rate (decreased motility) is an indication of damage to motor function[39,45,46]. We found that growing worms on 2% glucose media lowered the thrashing rate in wild-type worms (**Fig 4B**). The overexpression of tau (WT-Tau and A152T-Tau) alone is enough to significantly hinder the worm's thrashing rate at day 4 of adulthood (**Fig. 4B**). Glucose appeared to have no effect on WT-Tau worms and increased the thrashing rate of A152T-Tau worms, in contradiction to the effect of glucose on wild-type N2 worms, but consistent with glucose's ability to rescue the lifespan of the A152T transgenic worms (**Fig. 4B**). To confirm glucose's ability to hinder motility in control N2 worms, the average locomotive activity of animals, reared on control media or 2% glucose media, was measured using the WMicrotracker^{TM} (PhylumTech) (**Fig. 4 C**). Animals treated with glucose had significantly lower average activity units than those on normal media (**Fig. 4 C**). Worms that were grown on 2% glucose media had notably higher levels of triglycerides (**Fig. 4D**) demonstrating that a high glucose diet in *C. elegans* increases body fat content.

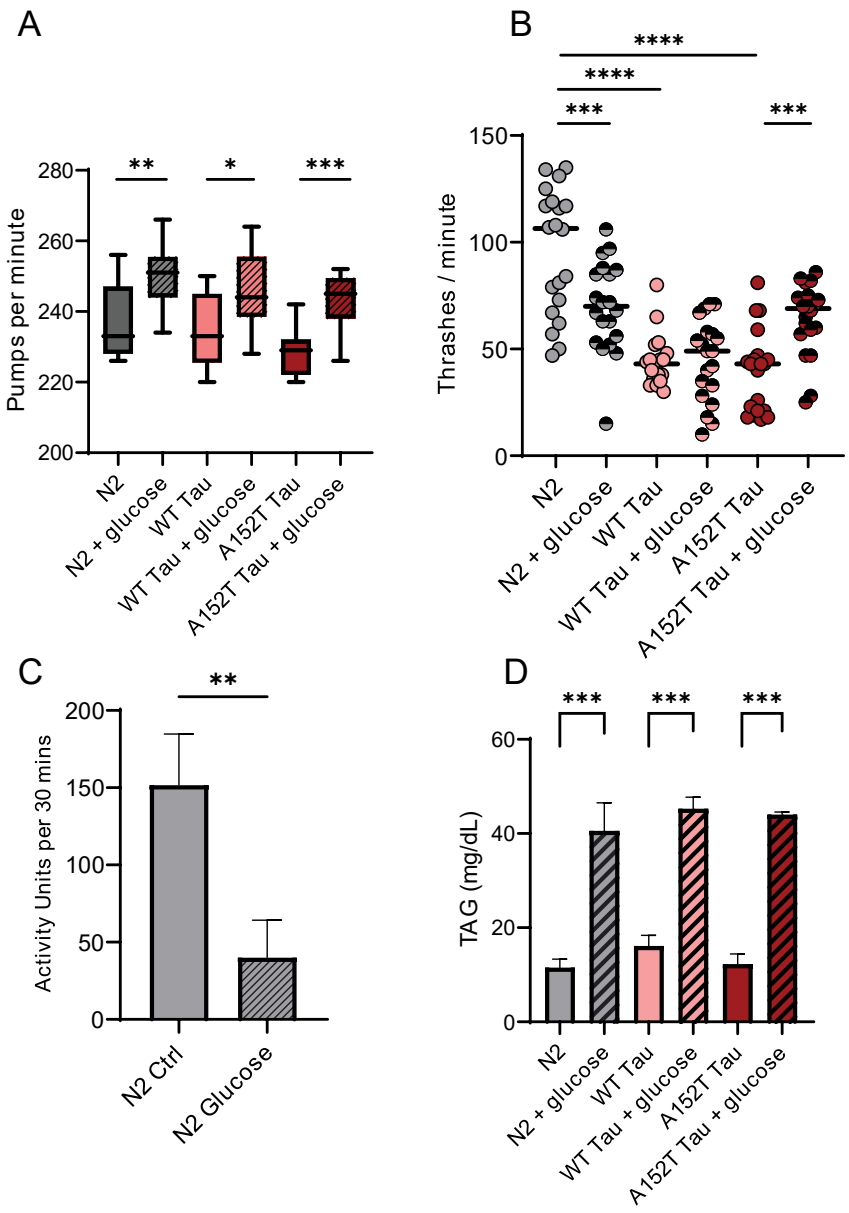

Figure 4: High glucose diet alters worm feeding behavior, motility, and triglyceride content

(A) Pumping assay for Day 2 adult animals grown on regular media or 2% glucose media. Pumping rate was quantified as the number of terminal bulb contractions over 30 seconds. n=12. **(B)** Thrashing assay analysis of day 4 adult animals grown on regular NGM media or 2% glucose media. **(C)** Control wild-type N2 animals were reared on either normal control media or 2% glucose media. Average locomotive activity was measured at the day 1 young adult stage using a WMicrotracker One™ (PhylumTech). **(D)** Analysis of triglyceride content in day 4 adult worms grown on regular media or 2% glucose media. Assay utilized the Stanbio LiquiColor Triglycerides kit, normalizing to protein content. **(A-D)** Statistics performed using one-way ANOVA with Sidak's multiple comparisons test.

Tau down regulates fat metabolism and glyoxalase gene expression in response to glucose

Previously, our lab found that the genes *fat-6*, *nhr-49*, and *mdt-15* were differentially expressed in *glod-4* mutant worms which accumulate AGEs. These genes are all involved in lipid metabolism, and the dysregulation of lipid pathways has been implicated in AD[47]. *nhr-49* (orthologue of human HNF4A and HNF4G) is a nuclear transcription factor that coordinates fat metabolism genes[48–50]. *mdt-15* (orthologue of human MED15) is an NHR-49 interacting protein and transcriptional coactivator[49,51,52]. *fat-6* (human orthologue of SCD5) is a stearoyl-CoA desaturase which is also important in regulating fat metabolism, and catalyzes the formation of monounsaturated fatty acids (MUFA) from saturated fatty acids (SFA)[53]. Given lipid metabolisms importance in AD, and these genes sensitivity to AGEs in *glod-4* mutants, we wished to observe if glucose could influence the lipid metabolism gene expression, and whether tau would alter that expression. In addition, we also wished to observe if glucose and tau might affect *glod-4* expression, which is responsible for detoxification of α-DCs. To do this, we performed an RT-qPCR experiment (**Fig. 5**). Animals were grown on either regular NGM or 2% glucose NGM and then collected at day 4 of adulthood. We found that N2 worms had a large increase in gene expression in all three lipid metabolism genes (**Fig. 5A-C)** indicating that the excess energy provided by the glucose was being converted to fat for storage and utilization. Glucose also increased *glod-4* expression in N2 worms (**Fig. 5D)** characteristic of an increased α-DC burden due to glucose treatment. Tau expressing animals, both WT-tau and A152T-tau, did not show this same transcriptional upregulation when stimulated with glucose, indicating that these strains have diminished responses in fat metabolism regulation and fatty acid biosynthesis, as well as glyoxalase activity (**Fig. 5).**

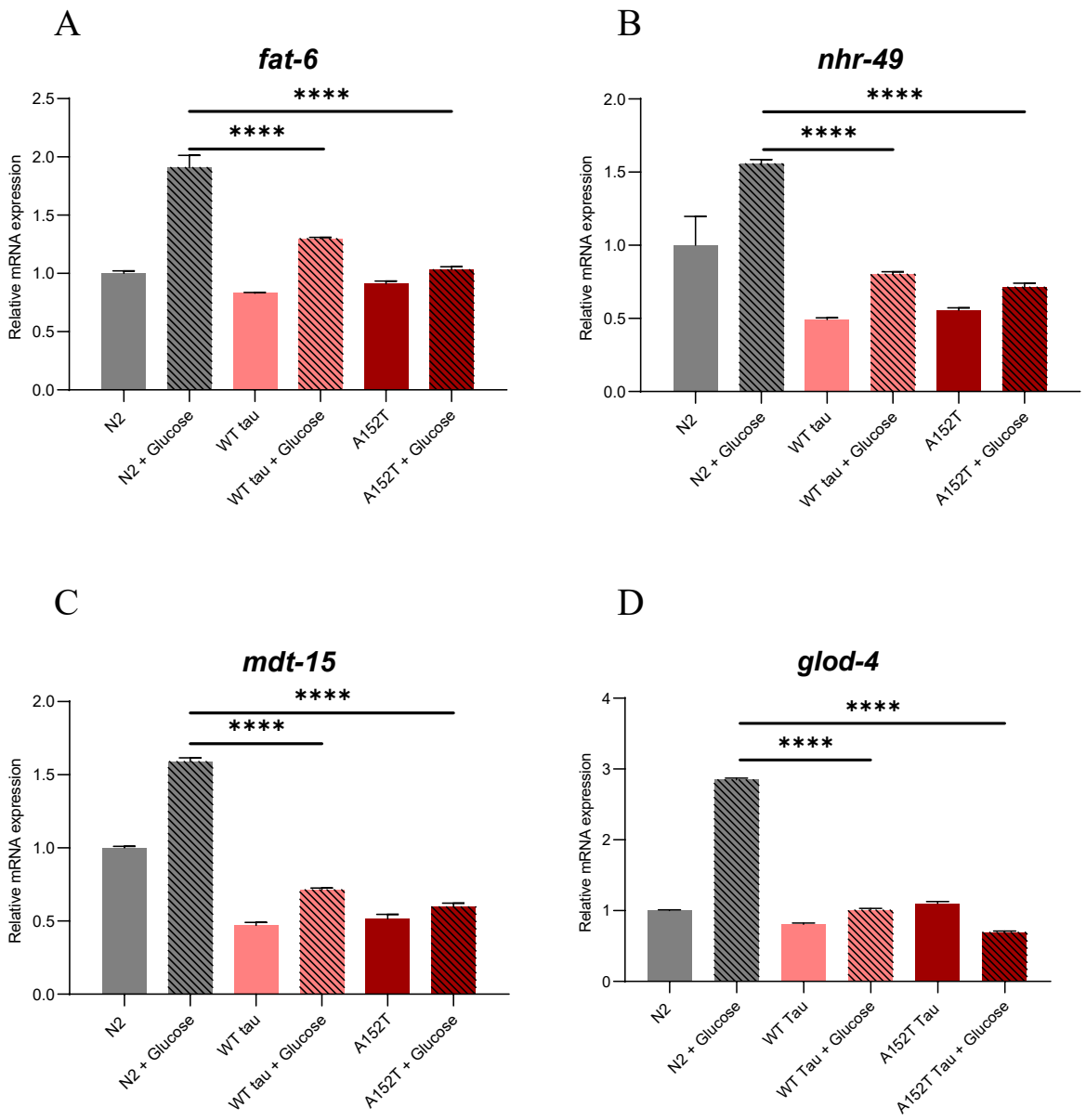

Figure 5: Fat metabolism and glyoxalase gene expression interrupted by tau.

Animals were grown on either regular NGM or 2% glucose NGM from young adult to Day 4 of adulthood then harvested. RNA was extracted and relative gene expression for *fat*-6 (**A**), *nhr*-49 (**B**), *mdt*-15 (**C**), and *glod-4* (**D**) determined with RT-qPCR. (**A-D**) All values are relative to N2 on regular control media. Statistics were determined using one-way ANOVA with Sidak's multiple comparisons test.

High glucose diet increases the accumulation of the AGE, MGH1, in *C. elegans*

Our hypothesis is that a high glucose diet could increase the accumulation of glycolysis derived AGEs, and that this increase in AGEs burden could be responsible for the health and behavior complications we have observed. To determine if a high glucose diet will increase the accumulation of AGEs in C. elegans, we harvested day 4 adult animals grown on either normal control media or 2% glucose media and performed a western blot where we probed for the AGE, methyl-glyoxal-hydroimidazolone (MGH1) (**Fig. 6**). Control N2 worms showed an increase in MGH1 intensity when treated with glucose (**Fig. 6B & C**). The WT-Tau expressing worms and the A152T-Tau expressing worms displayed a higher MGH1 accumulation than control N2 worms without any glucose treatment, and this high MGH1 accumulation remained relatively unchanged with glucose treatment (**Fig. 6B & C**). The 27 kDa band appears much more prominently in tau expressing animals (**Fig. 6C**).

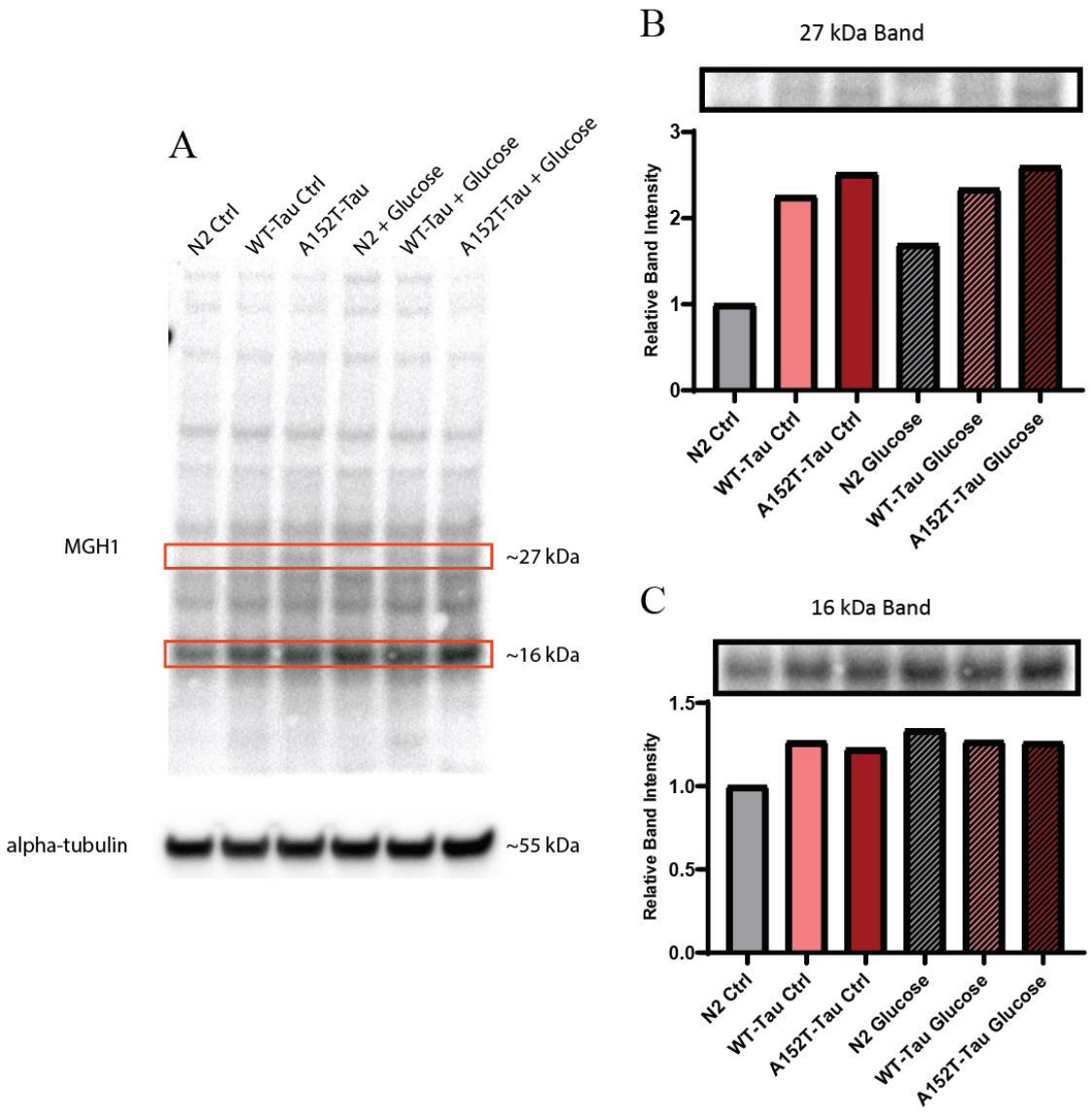

Figure 6: High Glucose diet increases the accumulation of MGH1.

Animals were harvested at day 4 of adulthood after being grown on either normal control media or 2% glucose media, protein was extracted, and western blot performed. (**A**) Membrane was probed for MGH1 and alpha-tubulin (for loading control). (**B**) Relative peak intensity of the 27 kDa band was calculated and normalized to N2 Ctrl. (**C**) Relative peak intensity of the 16 kDa band was calculated and normalized to N2 Ctrl.

MGH1 interacts with WT-Tau to decrease lifespan

To explore if the lifespan reduction seen with glucose treatment is due to the accumulation of AGEs, we treated C. elegans animals with MGH1 to observe if AGEs treatment can recapitulate the shortening of lifespan seen with glucose (**Fig. 7**). Only WT-Tau expressing

animals displayed a significantly shortened lifespan after treatment with MGH1 (**Fig. 7B**). Both wild-type N2 animals and A152T-Tau animals had no significant difference in lifespan after treatment with MGH1 (**Fig. 7A & C**).

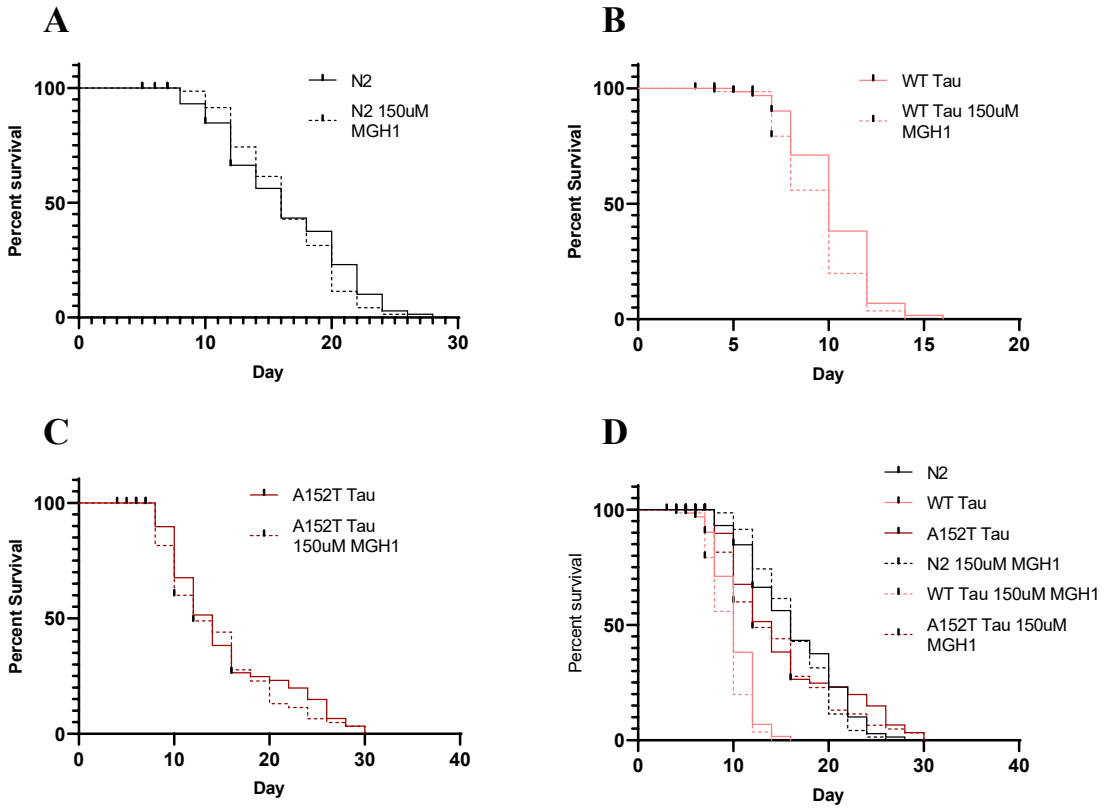

Figure 7: MGH1 treatment may interact with tau to decrease lifespan.

(**A**) Lifespan survival curve of control N2 animals grown on RNAi media with control L4440 vector bacteria, either with or without 150 µM MGH1 supplement. **(B)** Lifespan survival curve of WT-Tau expressing animals, either with or without 150 µM MGH1 supplement. (**C**) Lifespan survival curve of A152T-Tau animals grown, either with or without 150 µM MGH1 supplement. (**A-C**) Significance was determined using the log-rank (Mantel-Cox) test: WT-Tau compared to WT-Tau 150 µM MGH, P-value = 0.0221 (*).

MGH1 increases feeding behavior and decreases motility

We hypothesized that the increased feeding behavior and poor motility effects we saw upon glucose treatment (**Fig. 4**) could be driven in part by the increased accumulation of AGEs that glucose treatment causes (**Fig. 6**). Similar to what we saw with glucose treatment, animals

reared on 150 μM MGH1 displayed increased pumping rates compared to no treatment controls (**Fig. 8A**). Wild-type N2 animals appeared to trend toward lowered thrashing rates if treated with MGH1 (**Fig. 8B**), but this result was not statistically significant. To further analyze motility, day 1 young adult wild-type N2 animals reared on control media or 150 μM MGH1 media were measured for average locomotive activity using the WMicrotracker One^{TM} (PhylumTech). MGH1 treated animals displayed significantly lower activity units than those on control media (**Fig. 8C**).

Tau down regulates fat metabolism and glyoxalase activity in MGH1 response

To determine if gene expression changes seen with glucose treatment are from the accumulation of AGEs, RT-qPCR was performed after treatment with MGH1. Synchronized animals were grown on NGM with 100 μM MGH1 until day 4 of adulthood and compared to animals grown on control media (**Fig. 9**). Similar to glucose, in control N2 animals MGH1 upregulates *nhr-49* gene expression (**Fig. 9A**) as well as *glod-4* gene expression (**Fig. 9D**). Tau expressing animals, both WT-tau and A152T-tau, did not show this same transcriptional upregulation when stimulated with MGH1, indicating that these strains have diminished responses in fat metabolism regulation and glyoxalase activity.

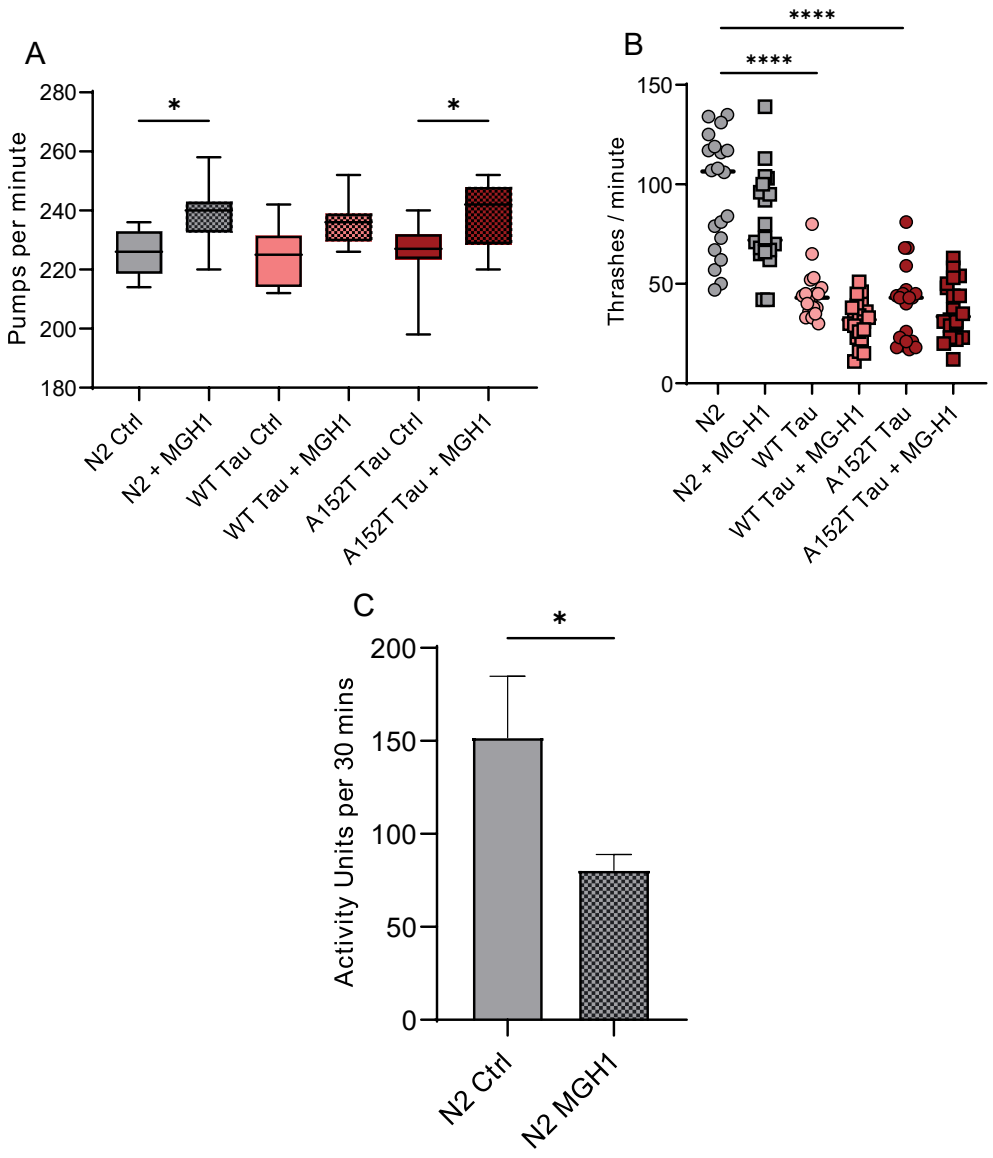

Figure 8: MGH1 treatment alters feeding behavior and mobility.

(A) Animals were reared on either normal control media or 150 µM MGH1 media. Pumping was measured at the young adult stage. (**B**) Animals were grown on either normal control media or 100 µM MGH1 media. Thrashing (body bending) was measured at day 4 of adulthood. (**C**) Animals were reared on either normal control media or 150 µM MGH1 media. Average locomotive activity was measured at the day 1 young adult stage using a WMicrotracker One™ (PhylumTech). (**A-C**) Statistics performed using one-way ANOVA with Sidak's multiple comparisons test.

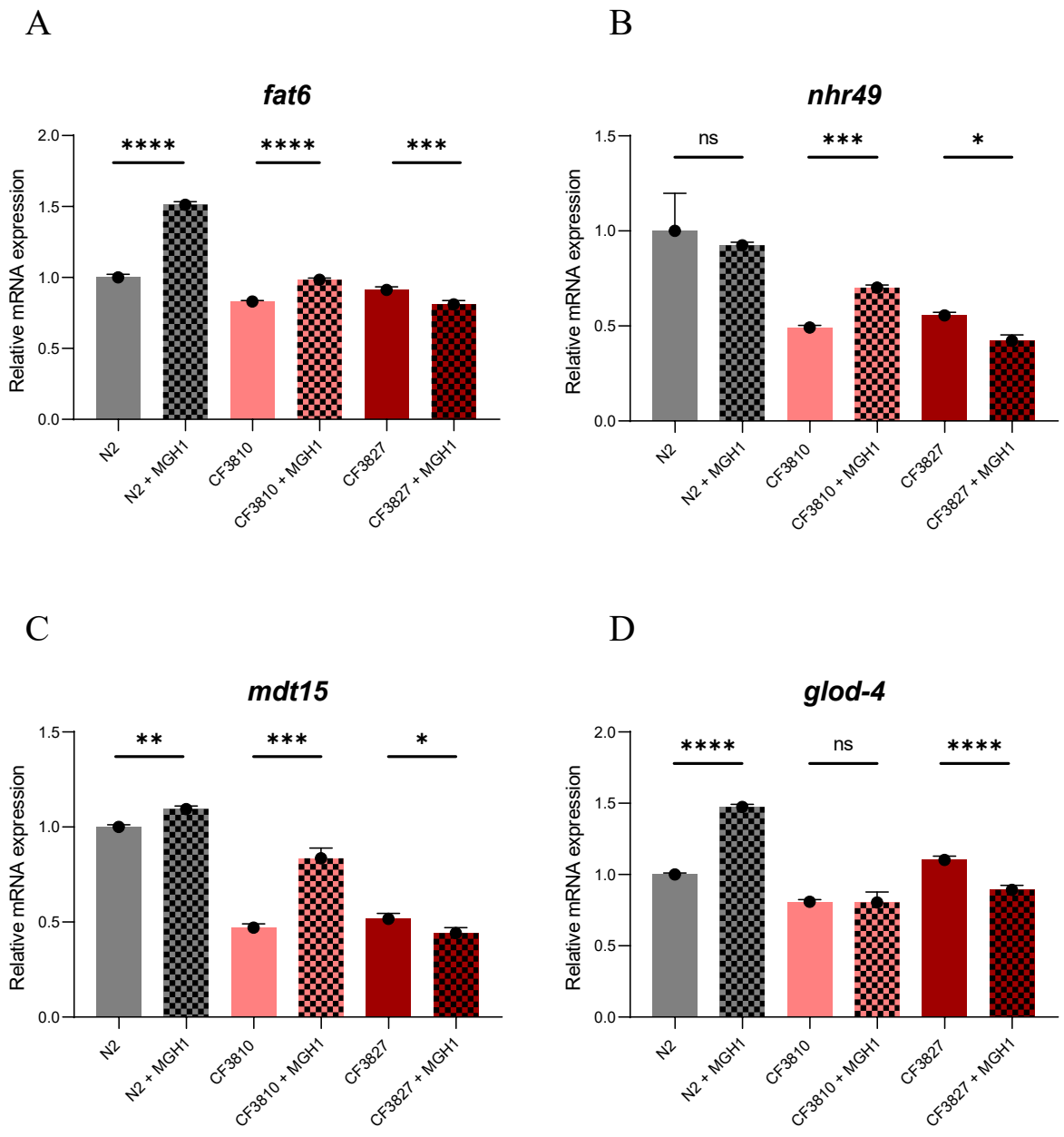

Figure 9: Tau interrupts gene expression in response to MGH1.

Animals were grown on either regular NGM or NGM with 100 µM MGH1 from young adult to Day 4 of adulthood then harvested. RNA was extracted and relative gene expression for *fat*-6 (**A**), *nhr-49* (**B**), *mdt-15* (**C**), and *glod-4* (**D**) determined with RT-qPCR. All values are relative to N2 on regular control media. Statistics were determined using one-way ANOVA with Sidak's multiple comparisons test.

MGH1 increases tau phosphorylation in human neuron cells

Previous studies have provided evidence that AGEs and the Receptor for Advanced Glycation Endproducts (RAGE) are implicated in increasing tau phosphorylation[54,55]. To determine if MGH1 can increase tau phosphorylation in human neurons *in vitro,* our collaborators Manish Chamoli, PhD and Cyrene Arputhasamy cultured human neurons SH-SY5Y and treated them with different levels of MGH1. Western blot probing for two different tau phosphorylation sites revealed an MGH1 dose dependent increase in tau phosphorylation (**Fig. 10**).

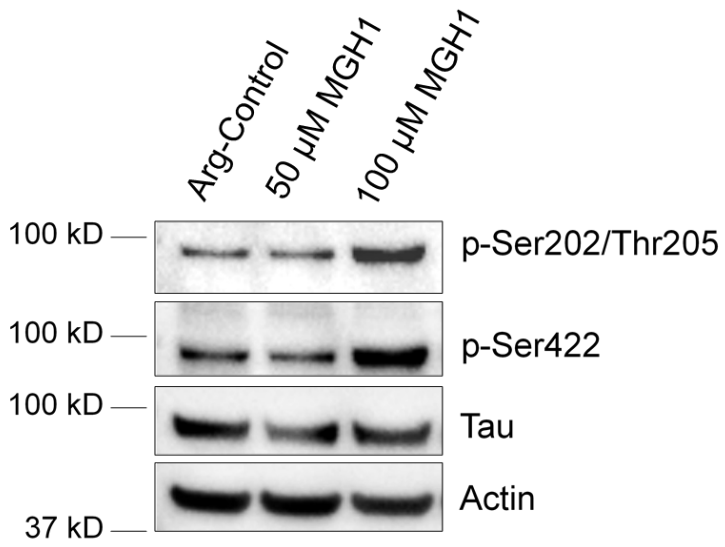

Figure 10: MGH1 increases tau phosphorylation in SH-SY5Y neurons.

Human neuronal SH-SY5y cells were treated with 50 µM MGH1, 100 µM MGH1, or arginine as a control. Western blot of protein extract was propped for tau and tau phosphorylation. Courtesy of Manish Chamoli, PhD and Cyrene Arputhasamy, Buck Institute for Research on Aging.

***tdc-1* RNAi knockdown does not rescue MGH1 toxicity**

Previously, our lab discovered that removing *tdc-1*, responsible for the biosynthesis of the neurotransmitter tyramine from the amino acid tyrosine, can rescue the reduced lifespan seen in glyoxalase mutants *glod-4* (**Fig. 2**). These glyoxalase mutants have a higher AGEs burden, and

so we wanted to know if knocking down *tdc-1* expression could improve the lifespan of animals treated with the AGE MGH1. We used *tdc-1* RNAi to knockdown *tdc-1* expression and grew animals on either control media or 150 µM MGH1 media (**Fig. 11A-C**). *tdc-1* RNAi knock down did not improve WT-Tau expressing animal lifespan compared to control RNAi vectors (**Fig. 11D**).

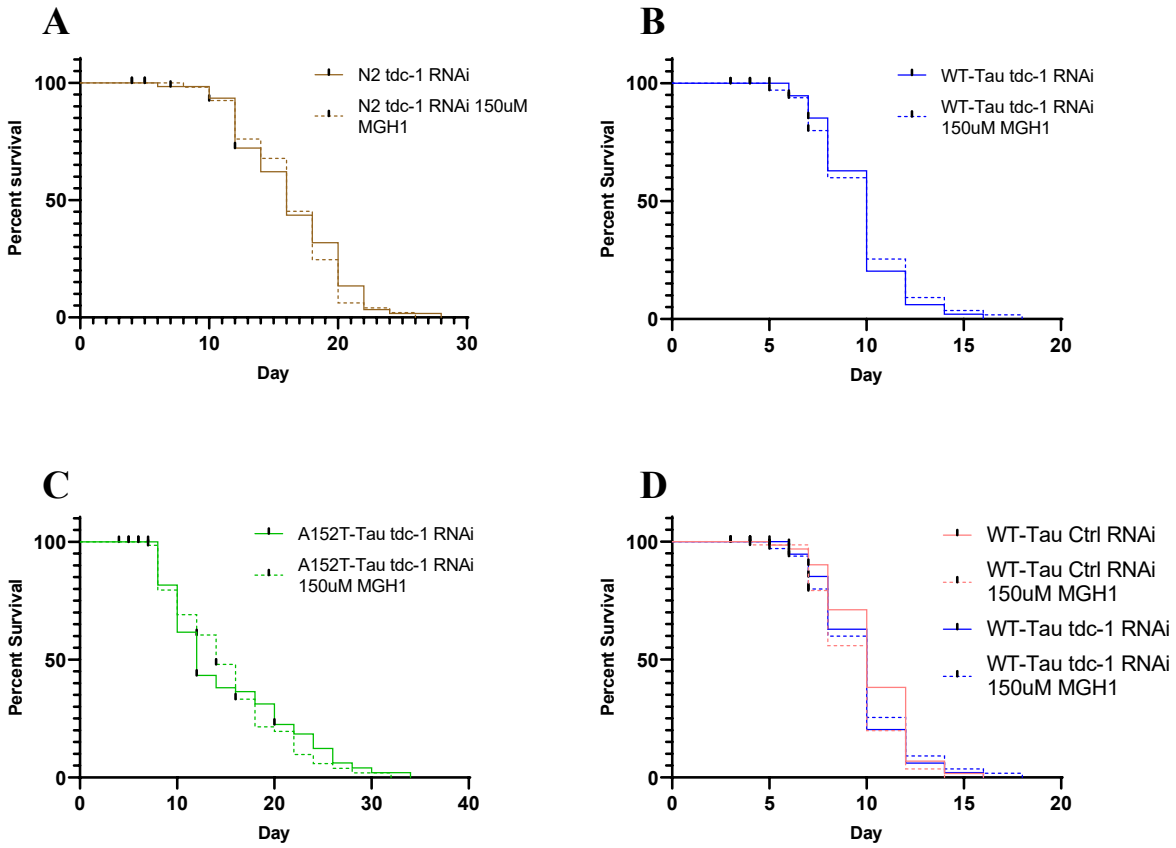

Figure 11: *tdc-1* RNAi knockdown does not rescue lifespan from MGH1 toxicity.

(**A-C**) Animals were grown on control media or 150 µM MGH1 media, with *tdc-1* RNAi bacteria. (**D**) Comparison of WT-Tau animals with either control RNAi bacteria or tdc-1 RNAi bacteria, and either control treatment or 150 µM MGH1 treatment.

Discussion

In spite of extensive studies in the field of diabetes mellitus the link between diabetes and Alzheimer's disease has been poorly explained[56,57]. This thesis argues that the connection between diabetes and AD (or other tauopathies) might originate from the by-products of glucose metabolism, specifically AGEs. Earlier reports demonstrated that these toxic compounds (AGEs) accumulate over age as a result of regular metabolism and dietary intake and cause irreversible changes in biological macromolecules[15,16,58,59]. Studying the effect of AGEs we observed interesting results that could profoundly increase our knowledge in the context of neurological disease like tauopathy.

Glucose rescue of A152T-Tau lifespan

The glucose lifespan experiments (**Fig. 3**) showed that the worms expressing human tau have shorter lifespans than control worms, with worms expressing A152T-Tau having the shortest lifespan. Adding glucose to the growth media reduced the lifespan of the wild-type control N2 worms and the WT-tau expressing worms, but interestingly, the lifespan of the A152T-Tau expressing worms improved when the animals were given glucose. We hypothesize that the A152T mutation may inhibit the metabolism of protein, or another non carbohydrate molecule, creating a energy deficit. Thus, providing additional glucose may rescue the A152T-Tau mutants from that energy deficit and rescue their lifespan.

MGH1 as the causative factor for feeding increase and decreased motility

We have shown that a high glucose diet causes an accumulation of AGEs (**Fig. 6**), and that treating animals with the AGE MGH1 can recapitulate the increased feeding and reduced motility seen with glucose (**Fig. 4A-C, Fig. 8**). This evidence suggests that glucose derived AGEs, such as MGH1, are the cause of the feeding and motility changes we observe. It's

possible that glucose and MGH1 affect feeding and motility in separate ways, but with similar outcomes; so further study is required to substantiate how glucose and MGH1 produce these effects.

Tau renders animals incapable of responding normally to glucose

It is not surprising that N2 control worms, given a high glucose diet, show an increase in expression of genes regulating fat metabolism (**Fig. 5A-C**). Since the glucose provides an excess of energy animals would be expected to convert some of that surplus into stored energy, i.e. fat. *nhr-49*, *mdt-15*, and *fat-6* all play a role in the regulation of fat metabolism and lipid biosythesis[49,51–53]. Fat metabolism involves the conversion of excess energy to long term fat/lipid stores, as well as the breakdown of those fat/lipid stores for use as energy. In contrast to control animals treated with glucose, transgenic tau animals do not show the same upregulation of these fat metabolism regulating genes (**Fig 5A-C**). This diminished response in fat regulation could have far reaching impacts on health. Recently, there has been mounting evidence that AD has links or similarities to metabolic disorders, such as impaired mitochondrial energy, brain insulin resistance, and dysfunctional lipid metabolism[3,4,60]. Our data, demonstrating that tau expressing animals do not process glucose normally (failing to engage lipid metabolism pathways as expected) supports the notion of AD as a metabolic disorder and places tau at the root of metabolic dysregulation. Additionally, with fat metabolism dysregulated, and an abundance of glucose in their diet, it is likely that even more glycolytic flux is occurring in these animals and more AGEs are being generated, a harmful feed forward loop in the generation of AGEs.

Tau dysregulates glyoxalase activity

Fat metabolism dysregulation was not the only gene expression dysregulation we observed in tau expressing animals. Control N2 animals exhibited an increase in glyoxalase

activity in response to glucose (**Fig. 5D**) as well as in response to MGH1 treatment (**Fig. 6D**). As glucose/glycolysis generates α-DCs, the upregulation of *glod-4/GLO1* is a natural response to detoxify the additional α-DCs being generated in a high glucose diet. The upregulation of *glod-4/GLO1* in control animals in response to MGH1 (**Fig. 6D**) indicates the glyoxalase pathway can also sense the accumulation of downstream AGEs.

Tau expressing animals however do not have the same *glod-4* response to glucose or MGH1 (**Fig. 5D & 9D**) While the WT-tau expressing animals do have an increase in *glod-4* expression in response to both glucose or MGH1, their response is noticeably diminished (**Fig. 5D & 9D**) compared to N2 animals. A152T animals have an even more severe dysregulation, even showing a decrease in *glod-4* gene expression after treatment with glucose or MGH1 (**Fig. 5D & 9D**). A diminished glucose response is interesting because it suggests that tau, likely aberrant hyperphosphorylated tau (**Fig. 10**), is somehow inhibiting glyoxalase expression.

Conclusion

Taken together our data indicate that tau expression, may cause a damaging disruption to glyoxalase expression and fat metabolism (**Fig. 5 & 9**). We postulate that this disruption likely promotes an increase in AGE accumulation which in turn can lead to further tau phosphorylation and aberration (**Fig. 10**). By interrupting fat metabolism (**Fig. 5 & 9**) tau expression likely promotes further reliance on glucose and glycolysis for energy, which we theorize would increase the generation of α-DCs and AGEs. Glyoxalases are important to detoxify α-DCs and curb the accumulation of AGEs, and by disrupting glyoxalase expression (**Fig. 5D & 9D**) tau could increase the accumulation of α-DCs and AGEs. With α-DCs and AGEs accumulating, tau phosphorylation increases (**Fig. 10**), likely contributing to AD hyperphosphorylated tau NFTs.

This also supports the building theory that Alzheimer's disease may be more closely associated with metabolic disorders.

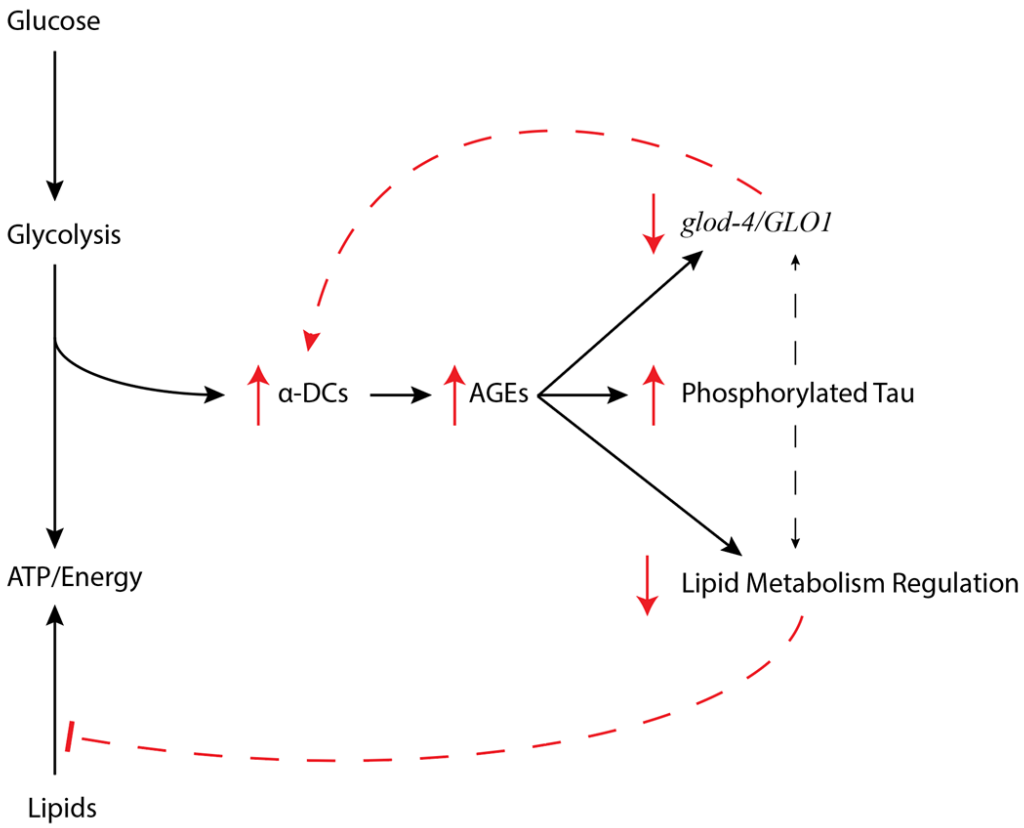

Figure 12: Graphical abstract depicting the effect of glucose on tauopathy

Red arrows depict harmful changes or interactions. Green arrows depict beneficial changes or interactions. Dashed lines represent suspected changes or interactions.

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