High Glucose Diet Attenuates Dopaminergic Neuronal Function in *C. elegans* Leading to Acceleration of Aging Process

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Abstract

Background: Environmental or exogenous factors that cause Parkinson's disease have not been sufficiently elucidated. Here, we investigate a causative effect of high glucose diet on Parkinson's disease-relevant dopaminergic neuronal system in C. elegans .

Methods: Aging parameters were first observed by measuring the lifespan, body movement, and body sizes with and without background of high glucose. Toxic effect of high glucose diet was further explored by observing the dopaminergic neurons using transgenic dat-1 ::GFP strains, BZ555 under Zeiss microscope. And extended the experiments by assessing dopamine-related behavioral analysis including basal slowing response and alcohol avoidance. Aggregation on the α -synucleins were also assessed by observing the NL5901 worms.

Results: . Worms fed with 250 mM glucose showed daf-2 -independent regulation of aging displaying short lifespan (≤15 days), long body size (max. 140%) and slow movement (min. 30%, 10 bends/min). Anterior dopaminergic neurons were rapidly inactivated (70%) by glucose-rich diet from 12 h of exposure suggesting specific degeneration in ADE neurons. The dysregulation of neurons led to deteriorations in dopaminergic behaviors including basal slowing response (BSR). High glucose diet decreased dopamine synthesis (40 vs. 15 pg/mg protein) and induced α-synuclein aggregation in the muscles.

Conclusion: Results demonstrate a potential of high glucose diet as a trigger of dopaminergic neuronal dysregulation conjugating aging acceleration.

Background

Aging is characterized by the loss of cellular function and the increase of vulnerability to environmental stress resulting in enhanced susceptibility to disease. In humans, aging increases risk for multiple chronic diseases including diabetes, Alzheimer's disease, Parkinson's disease. A susceptibility to the age-related disease has been widely investigated with regard to a connection with dietary behavior. In Asian countries, people's dietary habits tend to consume foods with high glycemic index (GI) values (Lin, 2010). High GI diet was linked with the development of cognitive impairment and dementia (Overman et al. 2017 and Dolan et al. 2018). Parkinson's disease is linked with the degenerative loss of neurons in the substantia nigra (Corti et al., 2011), and previous epidemiological studies proposed that high GI might be associated with Parkinson's disease development (Seaquist, 2010). Up to this date, despite extensive research about Parkinson's disease, proper treatments and mechanisms of its pathogenesis are still incompletely understood.

The use of model organism*, Caenorhabditis elegans* in aging research enables easy monitoring of age-related diseases since more than 83% of the genes are similar to human beings including those that are related with neurological diseases (Lai, 2000). Moreover, there have been *C. elegans* mutant strains for

Parkinson's disease study such as BZ555 of which GFP fluorophores are attached to dopamine transporter, DAT-1 on the dopaminergic neurons (Nass, 2002); NL5901 that expresses human α-synuclein in muscles together with yellow fluorescence protein (YFP) (van Ham, 2008); and worms with mutation in dopamine synthesis genes such as cat-2, cat-4 and bas-1 (Martinez et al., 2017). These worms exhibit Parkinson's disease-like symptoms including degeneration of dopamine neurons, loss of dopamine-related behaviors, deterioration in movements and aggregations of α-synuclein forming Lewy bodies.

Here, we investigated an interactive role of glucose-rich diet between aging and dopaminergic neuronal degeneration mechanism in *C. elegans* model organism using its transgenic mutants. This study also contributes to a further understanding of the progress of diabetes and Parkinson's disease during the aging process.

Methods

Experimental Model and Subject Details

C. elegans including Bristol N2 (wild type), CB1370 [daf-2(e1370) III], NL5901 (pkIs2386 [α-synuclein::YFP unc-119(+)]), BZ555 [egIs1 [dat-1::GFP], CB1112 [cat-2 (e112)] were obtained from the Caenorhabditis Genetics Center (CGC, at University of Minnesota, Minneapolis, MC, USA). Standard conditions were used for *C. elegans* propagation at 20°C. Worms were synchronized by hypochlorite bleaching, hatched overnight and were subsequently cultured on NGM plates with OP50. The synchronized worms were prepared and grown until the L4 larval stage.

Method Details

Analysis of lifespan - Life-span analysis of *C. elegans* was conducted following a previously described method with slight modification (Sutphin GL and Kaeberlein M, 2009). The test plates were prepared by adding 250 mM glucose to NGM plates before seeding with *E. coli* OP50. Lifespan was scored by transferring control worms and glucose-treated worms to a new control or glucose-containing plates, respectively. Nematodes are transferred every day during the reproduction stage and every other 3 days after the reproduction stage, until all worms are dead. Worms that are not moving are counted as dead while worms that crawled out of the plates were counted as missing. All trials were done three times and each trial has N ≥100 worms per group.

Locomotion assay - Ten synchronized L4 worms (strains N2, CB1370 and NL5901) were used to assess the locomotion of the nematodes. Treated worms were consequently incubated in the presence of food, with 250 mM glucose. To score the movement, plates were gently tapped to induce stimuli for the worms'

movement and waited for 2 s before counting the bends each worm would make. Bends were counted for 60 s. Locomotion assay was done until Day 5 of adulthood. At least three trials were conducted with N ≥20 worms per group.

C. elegans growth assay - Ten synchronized L4 worms (N2, CB1370 and NL5901) were used to measure the body sizes of both control and glucose-treated worms. Treated worms were consequently incubated in the presence of food, with 250 mM glucose. Prior to measurements, all nematodes were washed with an M9 buffer and were moved to fresh 35 mM NGM media. The worms' body size was estimated by microscopy (SZ61, Olympus, Japan) with digital camera (C-5050 zoom, Olympus, Japan). The body size of the individual worm was analyzed by using ImageJ software (http://imagej.nih.gov). Measurement of the worm's body size was done until Day 5 of adulthood. At least three trials were conducted with N ≥20 worms per group.

Dopaminergic neurons observation - Study of dopaminergic neurodegeneration was carried out by exposing the synchronized L4 BZ555 worms with 250 mM glucose at different time intervals 12 h, 24 h, and 48 h. After treatment, nematodes were washed with an M9 buffer to remove adhering bacteria on the bodies, then worms transferred to 2% agarose pads on glass slides mounted with 5 M Levamisole, and enclosed with a cover slip. Imaging of live (immobilized) worms was carried out to monitor the neurodegeneration by observing the green fluorescence (GFP) attached on the dopamine neurons using the fluorescence microscope, Axio Imager A2 (Carl Zeiss, Jena, Germany). All measurements were obtained at fixed fluorescence exposure time and were analyzed using ImageJ software. The tests were performed three trials each set. In a separate set of experiments, nematodes were treated with increasing concentration of glucose (50 mM, 100 mM and 250 mM) for 12 h. Again, after treatment, worms were mounted to an agarose pad as described above and were observed using Axio Imager A2 and analyzed using ImageJ software.

Basal slowing response - Basal slowing assays were done using a previously described (Chase et. al. 2004) with slight modification. The test plates were prepared by adding 250 mM glucose to NGM media before seeding with *E. coli* OP50. BSR was performed by transferring 30 worms from both the control and treated worms to a separate freshly prepared empty NGM media for exactly 5 minutes. After 5 minutes, half of the worms were then transferred to "with-food plate" (NGM media with seeded OP50 and the other half to "with no food-plate" (empty NGM media). Worms were allowed to acclimate to the assay plates for 2 min, and then the number of body bends/20 seconds was determined for each condition. Data was collected for 30 animals per condition. Percent slowing was calculated by dividing the difference between locomotion rates on and off food by the locomotion rate of food.

Quantitative Analysis of a-synuclein Accumulation - Accumulation of a-synuclein protein was measured in control and glucose-treated NL5901 worms. Synchronized NL5901 L4 larvae were cultured on E. coli OP50 NGM media, without or with increasing concentrations of glucose at 20ºC for 24 h. After treatment, nematodes were then transferred to 2% agarose pads on glass slides, mounted with 5 M Levamisole, and enclosed with a cover slip. Immobilized worms were observed and imaged using Axio Imager A2 to monitor the YFP signal which corresponds to the accumulation of the a-synuclein protein for the body region of each worm. The number of Lewy bodies were counted individually. .

Statistical Analysis

The comparison of experimental data from at least three independent experiments was conducted using a mean value with the error bar (standard deviation , ± S.D.), and the\statistical significance was determined by Student t-test (SigmaPlot 10.0, SPSS Inc., Chicago, IL, USA). When the p-values are less than 0.05 or 0.01 or 0.001, the data are considered statistically significant (*p□0.05, **p□0.01, and ***p□ 0.001).

Results

A. DAF-2-independent regulation of lifespan by high glucose diet

In the previous studies (Lee, 2009; Schlotterer et al., 2009; Choi, 2011; Liggett 2015), wildtype *C. elegans* (N2) grown on the medium containing high concentrations of glucose showed a shortened lifespan. In this study, the high concentration (250 mM) of glucose was toxic to the lifespan of wildtype *C. elegans*, approximately 50% of animals were dead at 8 days after adulthood (Fig. 1A). All of the worms fed with high glucose were dead before 15 days of adulthood, while some of animals without glucose feeding lived for 20 days in adulthood. In comparison with our previous study (Choi, 2011), the survival ratio of animals grown with 250 mM glucose much smaller than that of worms fed with 10-20 mg/L glucose, which demonstrates that the toxicity of glucose diet is exerted with dose-dependent manner.

Insulin-like growth factor receptor gene, DAF-2 is a regulator of *C. elegans* aging, and the mutation in DAF-2 showed a longer (approx. double) lifespan (Lee, 2009). In the present study, the mutant strain, daf-2 (e1370) lived longer than wildtype N2 strain in case of no glucose diets; however, the high glucose diet shortened the lifespan of this strain of mutation in DAF-2 (Fig. 1A). Given that the lifespan of *C. elegans* is governed by the genetic regulatory pathway with DAF-2 in the upstream (Lee, 2009), the high glucose diet-induced short lifespan in daf-2 (e1370) suggests a DAF-2-independent regulation of lifespan or aging in *C. elegans*. To discover a clue for explaining this DAF-2-independent aging regulation, we focused on

the animal behavior in the early adult stage (before 5 days of adulthood) without showing difference in survival ratio.

B. Deterioration of body size in early adulthood by high glucose diet

C. elegans' body size has been used as an indicator for the progress of aging or biological toxicity. Endogenous or exogenous conditions to accelerate aging of *C. elegans* led to the increase of body size while the uptake of toxic compounds resulted in the decrease of body size or suppression of body growth (Cha et al., 2012; Kim et al., 2017). During the investigation of *C. elegans* lifespan, a significant increase in their body lengths was observed in the worms fed with 250 mM glucose as early as on day 1 in adulthood (Fig. 1B). The body sizes of N2, CB1370 and NL5901 strains were 10%, 32% and 32% longer than those of worms without glucose diet, respectively. The high glucose diet-induced enlargement in body size was maintained until day 3 in adulthood (Data for Days 2 and 4, See Additional file 1). Considering that the increase of body size was enhanced in daf-2 mutant worms, the high glucose diet-induced acceleration of aging progress might be partially down-regulated by DAF-2. The high glucose diet showed a synergetic effect in NL5901 strain on the enlarged body size suggesting that the overexpression or aggregation of human α-synuclein protein in *C. elegans* might be more susceptible to high glucose consumption-induced acceleration of aging progress than wild-type animals. Results demonstrate that the high glucose diet might be associated with dopaminergic neurons or Parkinson's disease model. Therefore, we focused on the effect of high glucose diet on dopaminergic neuronal behaviors.

C. Slowness of body movement by high glucose diet

A slowness of body movement is one of representative behavior accompanying aging and Parkinson's disease (Chase et. al. 2004), which was observed in the worms fed with the high glucose diet. Although the survival ratio of worms fed with high glucose diets was similar to control animals, the slowness of body movement was significantly induced by 250 mM glucose in the early adulthood (day 3) (Fig. 1C). The wildtype N2 worms without the high glucose diet showed the intact body movement (45-50 bends/min) at 3-5 days of adulthood while the body movement of animals fed with 250 mM glucose significantly decreased (20-30 bends/min) at the same period. Such high glucose diet-induced movement slowness was also found in the daf-2 mutant strain (CB1370) at the same period of adulthood, which implies that the shortened lifespan and acceleration of aging behavior by 250 mM glucose is independent of DAF-2 regulatory pathway. The high glucose diet drastically accelerated the progress of body movement slowness in the NL5901 strain expressing human α-synuclein from the day 1 in adulthood. These results suggest that the DAF-2-independent lifespan dysregulation might be highly associated with dopaminergic neuron degeneration and genetic regulatory pathway for Parkinson's disease.

D. Degeneration of dopaminergic neurons by high glucose diet

To identify whether the slowness of body movement in the animals fed with high concentration of glucose was linked with degeneration of dopaminergic neurons, the fluorescence from dat-1::gfp strain (BZ555) was examined under the microscope. DAT-1 gene is specifically expressed in the dopaminergic neurons and involved in the transportation of dopamine between neuronal cells (Sulston, 1975). Before the onset of body movement slowness by high glucose diet, the dopaminergic neurons of *C. elegans* fed with 250 mM glucose were significantly inactivated or degenerated with the significant reduction of DAT-1::GFP fluorescence intensity at 12, 24 and 48 h of adulthood (Fig. 2A). Quantitative analyses using ImageJ computer program revealed that dopaminergic neurons of worms fed with 250 mM glucose showed 20-40% loss of fluorescence intensity, suggesting inactivation or degeneration of dopaminergic neurons (Fig. 2B).

The anterior part or head of *C. elegans* contains two types of dopaminergic neurons, ADE and CEP (Sulston, 1975). To identify which neuron was predominantly degenerated by a high glucose diet, we observed the fluorescence of each type of dopaminergic neuron by separating ADE and CEP (Fig. 2C). In particular, the fluorescence level of the ADE neuron was dominantly diminished with increase of glucose concentrations, suggesting that a high glucose diet led to the degeneration of the ADE neuron. Quantitative analyses using ImageJ computer program revealed that about 80% of DAT-1 was inactivated in the worms fed with 250 mM glucose at 12 h of adulthood (See Additional file 2). The results suggest that ADE neurons are more susceptible to a high glucose diet than CEP neurons in dopaminergic neuronal systems in *C. elegans.*

E. CAT-2-independent dysregulation of dopaminergic neuronal behavior

The degeneration or inactivation of dopamine neurons in *C. elegans* deteriorated animals' dopamine-related behaviors including the basal slowing response (BSR) and alcohol avoidance. When *C. elegans* are placed on the agar plate in the presence of bacterial food, the animals show the slower crawling locomotion compared with them in the absence of bacterial food (Chase et. al. 2004). Such locomotion to become slow is defined as BSR and regulated by dopaminergic neurons in *C. elegans*. The defectiveness in the dopamine signaling hinders the ability of the worms to slow down in the presence of food resulting in similar or faster crawling speeds compared with them in the absence of bacterial food. Wild-type N2 worms without high glucose diet showed slower locomotion when placed on the (+) food plates compared with worms on the (-) food plates (% BSR: 60.98, Fig. 3A) (Additional files 3 to 6). However, high glucose (50, 100 and 250 mM) diets led to the decrease of BSR percentages with values 5.75%, 1.03% and -2.87%, respectively. The speed of body bends in *C. elegans* fed with high

concentrations of glucose was not reduced when they were placed in the presence of bacterial food. There was no difference in body bends between with food and without food in case of worms fed with high concentrations glucose (Fig. 3A). These results indicate that the high glucose diet-induced deterioration of dopaminergic neuronal system, dopamine synthesis and transport, disables worms' responsible locomotion.

Since the BSR behavior is governed by dopaminergic neuronal system, the BSR is significantly diminished when their dopamine is hardly produced by the mutation in CAT-2, a tyrosine hydroxylase, an enzyme necessary for dopamine synthesis in *C. elegans* (Sulston et al., 1975). The BSR percentage significantly decreased down to 12.6% in cat-2 (e1112) mutant worms from 61.0% (wild-type N2) without a high glucose diet (Fig. 3B). The percentage of BSR in cat-2 (e1112) was reduced from 12.6% to 4.8% and 6.4% in the worms fed with 100 and 250 mM glucose, respectively (Fig. 3B). Given that there are alternative pathways to produce dopamine or dopamine-like neurotransmitters, these results suggest a high glucose diet-induced suppression of dopamine synthesis or transport. These results also support the less fluorescence of DAT-1::GFP indicating inactivation of dopamine transport in *C. elegans* fed with high concentrations of glucose.

F. Decrease of in vivo dopamine level by high glucose diet

Wild-type N2 worms with high glucose diet showed cat-2 mutant-like, dopamine-depletion behaviors. To verify whether the dopamine contents decrease in *C. elegans* with a high glucose diet, the quantitative measurement of in vivo dopamine level was performed by HPLC analysis using whole body extracts from worms at 1 day adulthood. The dopamine level of high glucose diet N2 worms was about 15 pg/mg protein, which was 35% of dopamine content in the control N2 group (42 pg/mg protein, without glucose diet) (Fig. 4). This result indicates that the glucose consumption (250 mM) significantly inhibits dopamine synthesis in *C. elegans*. The dopamine transporter, DAT-1 was significantly deactivated in BZ555 strain worms with 250 mM glucose at 12 h (Fig. 2C). However, the dopamine contents were not significantly different between control group (0 mM glucose) and high glucose diet group (250 mM glucose) in dat-1::gfp strains, BZ555 (Fig. 4). These results demonstrate that high glucose diets degenerate dopaminergic neuronal systems by reducing dopamine transport through DAT-1 despite the same or similar dopamine level in the body.

G. Aggregation of α-synuclein by high glucose diet

In human Parkinson's disease model, α-synuclein, presynaptic cytoplasmic protein is reported to regulate dopamine transport (Nemani et al., 2010). However, the aggregation of α-synuclein to form Lewy bodies is highly linked to the progress of Parkinson's disease by dysregulation of the dopaminergic system. To

investigate the effect of high glucose diet on α-synuclein aggregation, NL5901 strain worms (Hamamichi et al., 2008; van Ham et al., 2008; Bodhicharla et al., 2012) in which human α-synuclein fused with yellow fluorescent protein (YFP) is expressed in body wall muscle were observed under the fluorescence microscope. When the glucose concentration increased from 50 to 250 mM, the number of visible α-synuclein aggregates increased from 20 to 60 by dose-dependent manner (Fig. 5). Considering *C. elegans* as a human surrogate system, this result demonstrates that the high glucose diet has a possibility to cause a dysregulation of dopamine synthesis and transport through abnormal and enhanced α-synuclein aggregation.

Discussions

The linkage of several neurodegenerative diseases including Parkinson's disease with Type 2 diabetes is one of the most discussed researches (Rulifson et al., 2002). Previous studies have shown that individual's high glycemic index are more prone to developing such diseases, suggesting a high prevalence of insulin resistance in Parkinson's disease patients (Schernhammer et., 2011). However, the association mechanism between Parkinson's disease and insulin signaling pathway remains unknown up to this date. Here we suggest that high glucose diet causes DAF-2-independent regulation of lifespan in *C. elegans*. Given that DAF-2 is an insulin receptor family protein localized on the very upstream part, the onset of Parkinson's disease-related aging might be regulated by the more upstream factor than DAF-2 or by an alternative pathway that is eventually merged into the insulin signaling. Our results of DAF-independent body movement and body size increase also support the existence of alternative pathways stimulated by high concentration glucose. Further studies about the effect of high glucose diet on toll-like receptors and downstream regulators of insulin and IGF-1 signaling (IIS) pathway are required to elucidate the mechanism explaining our results.

Among the dopaminergic neurons in *C. elegans*, ADE neurons were particularly inactivated with less fluorescence intensity from DAT-1::GFP than in the worms exposed to high concentrations of glucose in comparison with CEP neurons. Given that CEP neurons are responsible for the food recognition, the stronger fluorescence intensity of CEP neurons indicates that the defectiveness in basal slowing response (BSR) behavior was caused by low dopamine level or transport of dopamine, not by food recognition (Fig. 3). Unlike CEP neurons, ADE neurons contain tyramine receptors in addition to dopamine receptors, and tyramine is a precursor or alternative of dopamine and acts as a catecholamine releasing agent. Therefore, worm's dopaminergic behaviors including BSR and alcohol avoidance are more deteriorated by high glucose diet-induced specific and early degeneration of ADE neurons. The more deterioration of ADE than CEP was also found in the previous study (Salim and Rajini, 2016) focusing on a synergetic effect of glucose-rich diet on insecticide-induced dopaminergic neuronal dysfunction in *C. elegans*. Without insecticides, we here found that high glucose diet independently induced dopaminergic neuronal dysfunction *in C. elegans* through the reduction of dopamine synthesis and transport.

Recent studies about Parkinson's disease revealed that α-synuclein propagation was modulated in *C. elegans* and mouse models by Parkinson's disease-linked kinases activity-dependent manner (Bae et al, 2018). Given that the glucose-rich diet specifically degenerated ADE neurons containing the D2 class dopamine receptor (Kotecha et al, 2002), receptor tyrosine kinases should not be transactivated in *C. elegans*. Such deterioration in protein kinase activity presumably affects Parkinson's disease-linked kinases including LRK-1, an ortholog of human LRRK1 (leucine rich repeat kinase 1) and results in more α-synuclein aggregates in anterior muscles of worms fed with glucose-rich diet (Fig. 5) in comparison with control group without glucose. Although a further study about the effect of glucose on LRK-1 activity is necessary, the diminished activities of both DAT-1 protein and ADE neurons suggest a possibility of protein kinase dysregulation in *C. elegans*.

Conclusion

In this paper we discussed how high glucose diet could eventually lead to degeneration of dopaminergic neurons.

In conclusion, a high glucose diet accelerates aging progress along with body size increase and body movement decrease in *C. elegans* by daf-2-independent manner. The abnormal aging process is strongly linked with Parkinson's disease footage including dopamine level reduction, defective BSR, DAT-1 degeneration and α-synuclein aggregation as shown in the working model (Fig 6). Thus, the control of glucose consumption has the potential for slowing aging and neurodegenerative disease progression.

Abbreviation

PD –Parkinson's Disease

BSR – Basal Slowing Response

YFP – Yellow fluorescence protein

GFP – Green fluorescence protein

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for Publication

Not applicable.

Availability of supporting data

The datasets supporting the conclusions of this article are included within the article (and its additional file(s)).

Competing interests

The authors declare that they have no competing interests.

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Authors contribution

A.C.V.dG. and S.S.C. designed the experiments. A.C.V.dG. performed C. elegans cultures and assays. E.J.K. performed the HPLC analysis. J.H.C. and J.H.K. guided A.C.V.dG. for microscopic analysis. S.S.C. and A.C.V.dG. wrote manuscript. All authors reviewed and contributed to the manuscript. All authors read and approved the final manuscript.

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Additional Files

Additional file 1 — **Increase in the body size of all strains upon 250 mM glucose feeding. Increase** in the body sizes were observed from days 1 to 3 and levels off on Day 4 and 5. Sudden increase in the body size indicates advanced aging.

Additional file 2 — **Glucose concentration-dependent and neuronal cell-specific degeneration of the dopaminergic neurons.** Worms were exposed to different concentrations of glucose ranging from 50 to 250 mM for 12 h to observe the degradation of dopaminergic neurons. Among dopaminergic neurons, ADE neurons were more dominantly degenerated by high glucose diet.

Additional file 3 — **Video 1. Basal Slowing Response of control N2 worms (not treated with glucose diet; "non-food" plate).** During the BSR assay, worms that were not treated with glucose exhibited normal body movement when placed in a "non-food plate"

Additional file 4 **— Video 2. Basal Slowing Response of control N2 worms (not treated with glucose diet; "on-food" plate).** During the BSR assay, worms that were not treated with glucose tend to slow down as they approach the bacterial lawn. Slower body movements of C. elegans was recorded when placed in an "on-food plate".

Additional file 5 — **Basal Slowing Response of glucose-treated N2 worms (treated with glucose diet; "non-food" plate).** During the BSR assay, worms that were not treated with glucose exhibited normal body movement when placed in a "non-food plate".

Additional file 6 — **Video 4. Basal Slowing Response of glucose-treated N2 worms (treated with glucose diet; "on-food" plate).** During the BSR assay, worms that were treated with glucose did not exhibit any change on their movements as they approach the bacterial lawn. There were no significant changes in the worms' body movements on- and off- food were recorded when placed in an "on-food plate".

Figures

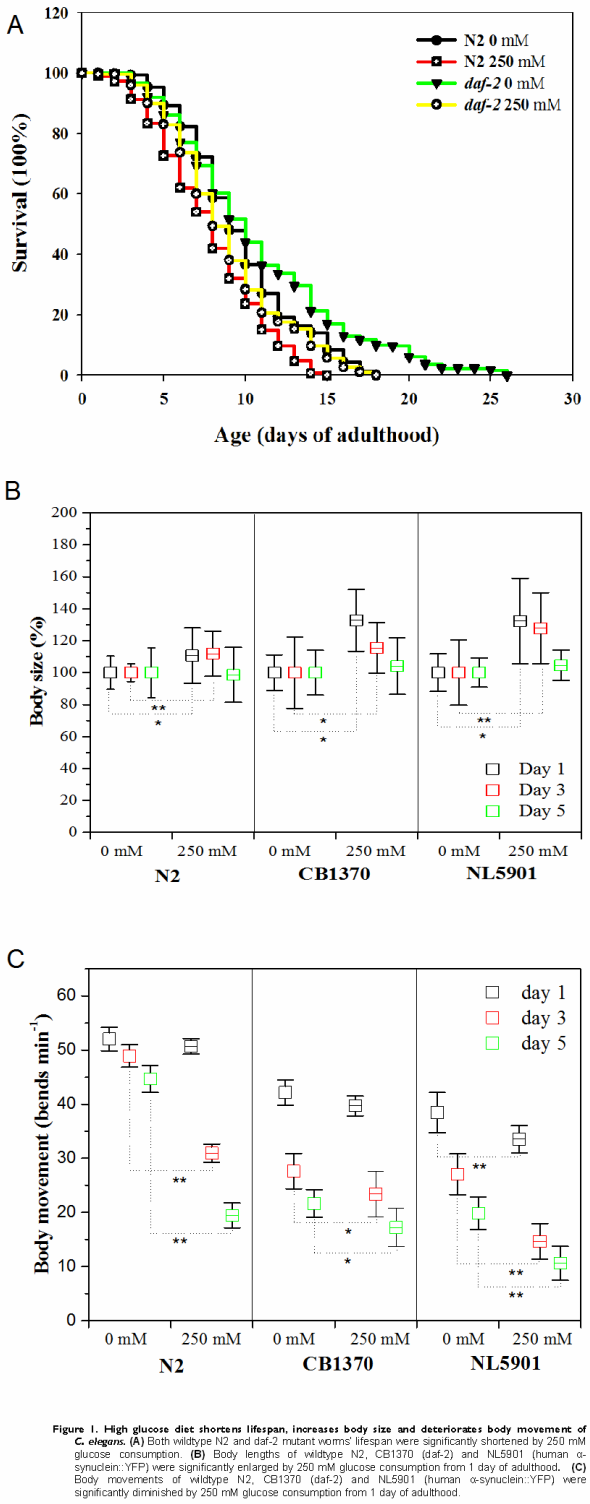

Figure 1. High glucose diet shortens lifespan, increases body size and deteriorates body movement of *C. elegans*. **(A)** Both wildtype N2 and daf-2 mutant worms' lifespan were significantly shortened by 250 mM glucose consumption. **(B)** Body lengths of wildtype N2, CB1370 (daf-2) and NL5901 (human α-synuclein::YFP) were significantly enlarged by 250 mM glucose consumption from 1 day of adulthood. **(C)** Body movements of wildtype N2, CB1370 (daf-2) and NL5901 (human α-synuclein::YFP) were significantly diminished by 250 mM glucose consumption from 1 day of adulthood.

Figure 1

High glucose diet shortens lifespan, increases body size and deteriorates body movement of C. elegans. (A) Both wildtype N2 and daf-2 mutant worms' lifespan were significantly shortened by 250 mM glucose consumption. n=100 (B) Body lengths of wildtype N2, CB1370 (daf-2) and NL5901 (human α-synuclein::YFP) were significantly enlarged by 250 mM glucose consumption from 1 day of adulthood. n=20 * P>0.05; **P>0.001 (C) Body movements of wildtype N2, CB1370 (daf-2) and NL5901 (human α-

synuclein::YFP) were significantly diminished by 250 mM glucose consumption from 1 day of adulthood. n=20 * P>0.05; **P>0.001

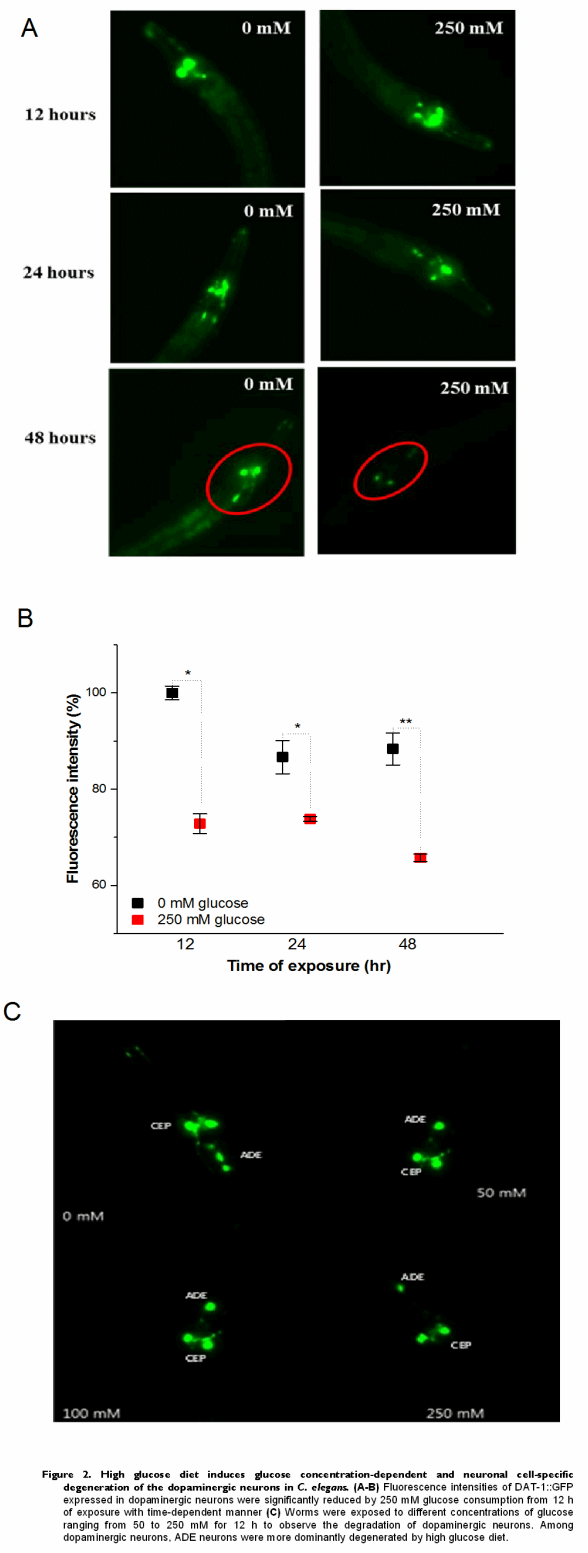

Figure 2. High glucose diet induces glucose concentration-dependent and neuronal cell-specific degeneration of the dopaminergic neurons in *C. elegans*. (A-B) Fluorescence intensities of DAT-1::GFP expressed in dopaminergic neurons were significantly reduced by 250 mM glucose consumption from 12 h of exposure with time-dependent manner **(C)** Worms were exposed to different concentrations of glucose ranging from 50 to 250 mM for 12 h to observe the degradation of dopaminergic neurons. Among dopaminergic neurons, ADE neurons were more dominantly degenerated by high glucose diet.

Figure 2

High glucose diet induces glucose concentration-dependent and neuronal cell-specific degeneration of the dopaminergic neurons in C. elegans. .. Fluorescence intensities of DAT-1::GFP expressed in dopaminergic neurons were significantly reduced by 250 mM glucose consumption from 12 h of

exposure with time-dependent manner. (A-B) Worms were exposed to different concentrations of glucose ranging from 50 to 250 mM for 12 h to observe the degradation of dopaminergic neurons. Among dopaminergic neurons, ADE neurons were more dominantly degenerated by high glucose diet. (C). n=10 * P>0.05; **P>0.001

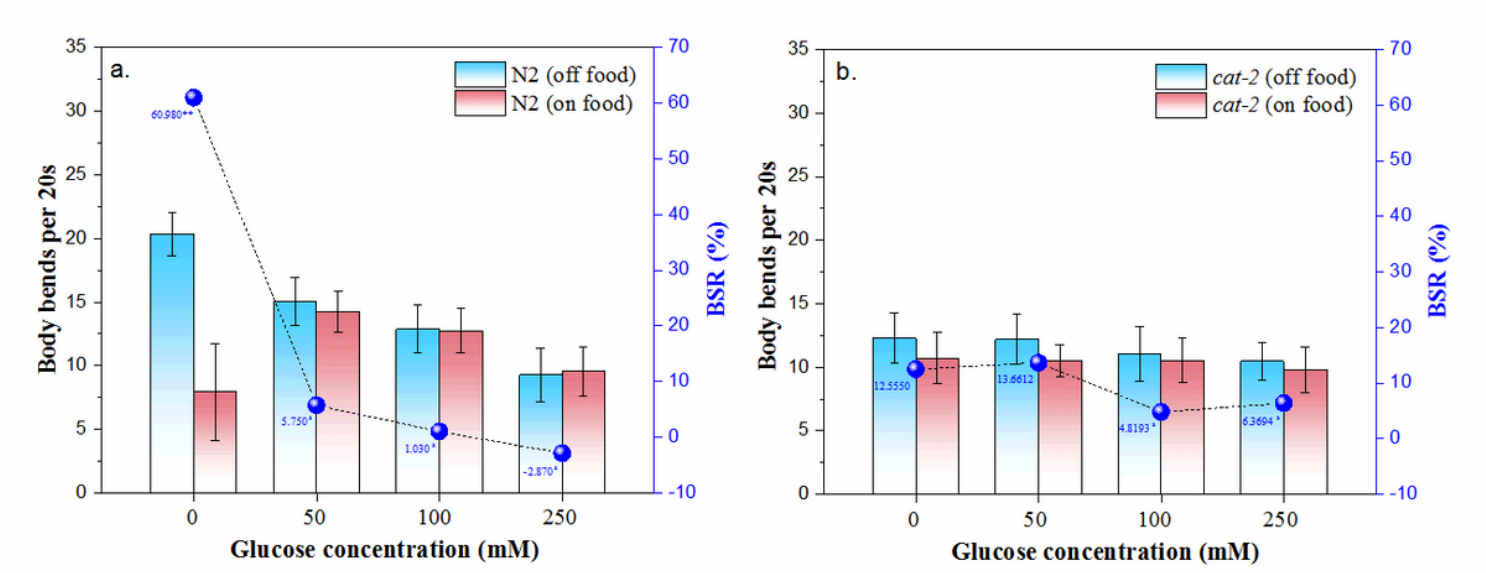

Figure 3

High glucose diet deteriorates dopaminergic behavior, basal slowing response (BSR). Wild type N2 worms exhibited loss of BSR upon feeding of increasing concentration of glucose (A). The cat-2 (e1112) mutant worms, on the other hand, failed to exhibit BSR but upon further exposure to glucose, %BSR also decreased (B). n=30 * means exhibited slight BSR A means defect in BSR compared to wild type

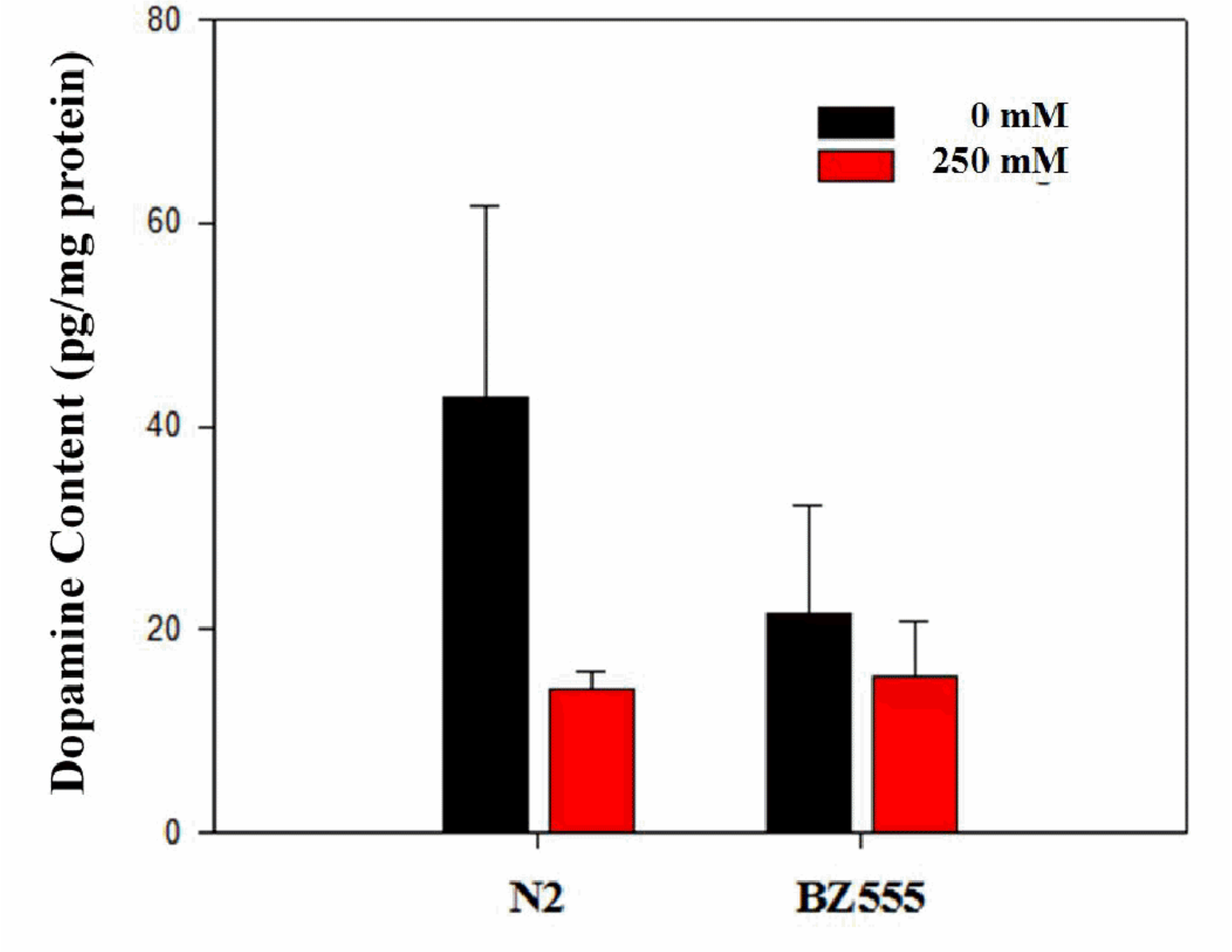

Figure 4

High glucose diet reduces dopamine synthesis level of C. elegans. Total dopamine contents measured using HPLC analysis were significantly reduced in wildtype N2 and slightly in BZ555 (cat-2) by 250 mM glucose consumption.

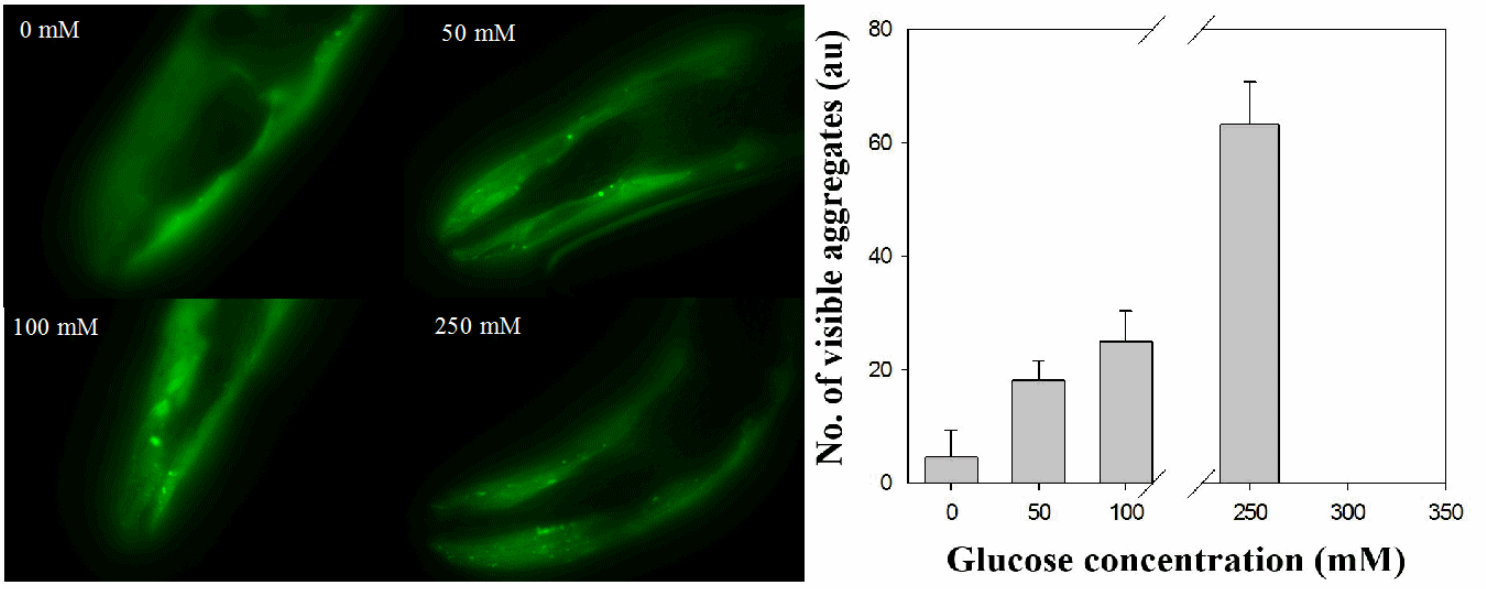

Figure 5

High glucose diet induces and increases α-synuclein aggregation in muscles of C. elegans. Number of α-synuclein aggregates were drastically increased in NL5901 worms expressing human α-synuclein by dietary glucose concentration-dependent manner.

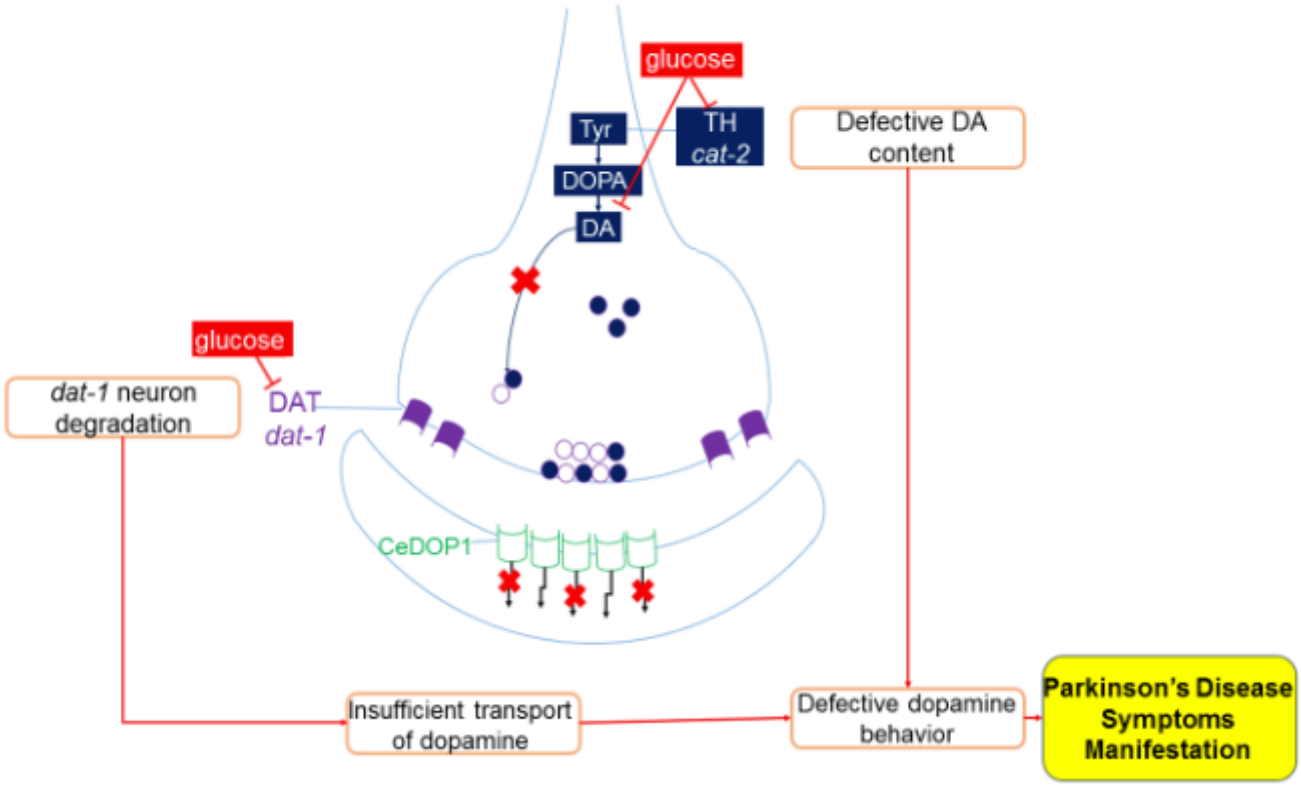

Figure 6

A working model of high glucose diet-induced Parkinson’s disease symptoms in C. elegans.

Supplementary Files

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- Additionalfile6.250mMONFOOD.mp4.m4v
- Additionalfile5.250mMN2NONFOOD.m4v
- Additionalfile3.0mMN2NONFOOD.m4v
- Additionalfile4.0mMN2ONFOOD.mp4
- GraphicalAbsract.png
- AdditionalFile1.BODYSIZE.jpg
- SupplementaryInformation.docx