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

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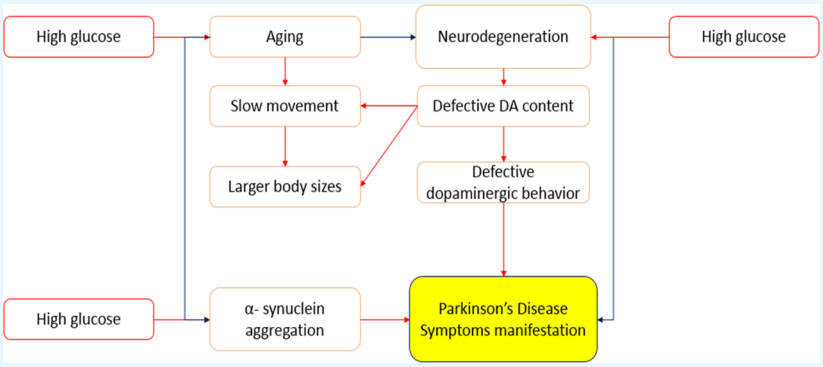

ABSTRACT: Parkinson's disease (PD) is a neurodegenerative disease characterized by the selective degeneration of neurons, primarily in the substantia nigra. Environmental or exogenous factors that cause Parkinson's disease have not been sufficiently elucidated. Our study aims to investigate the causative effect of a high-glucose diet on Parkinson's disease-relevant dopaminergic neuronal system in *Caenorhabditis elegans*. Aging parameters were first observed by measuring the lifespan, body movement, and body sizes with and without the background of high glucose. The toxic effect of a high-glucose diet was further explored by observing the dopaminergic neurons using transgenic Pdat-1::gfp strains, BZ555, under a Zeiss microscope, and the experiments were extended by assessing dopamine-related behavioral analysis including basal slowing response and alcohol avoidance. The aggregation of the α-synucleins was also assessed by observing the NL5901 mutants. Worms fed with 250 mM glucose showed *daf-2*-independent regulation of aging, displaying a 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 a 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). A high-glucose diet decreased dopamine synthesis (40 pg/mg vs 15 pg/mg protein) and induced α-synuclein aggregation in the muscles. Results demonstrate the potential of a high-glucose diet as a trigger of dopaminergic neuronal dysregulation conjugating aging acceleration.

1. INTRODUCTION

Aging is characterized by the loss of the cellular function and the increase of vulnerability to environmental stress, resulting in enhanced susceptibility to disease.[1] In humans, aging increases the risk for multiple chronic diseases including diabetes, Alzheimer's disease, and Parkinson's disease.[2] Susceptibility to age-related disease has been widely investigated regarding a connection with dietary behavior. In Asian countries, people's dietary habits tend to consume foods with high glycemic index (GI) values.[3] A high GI diet was linked with the development of cognitive impairment and dementia.[4,5] Previous epidemiological studies also proposed that high GI might be associated with Parkinson's disease development.[6]

Parkinson's disease is linked with the degenerative loss of the dopamine (DA) neurons in the substantia nigra.[7] This neuronal loss was found to contribute to the motor symptoms of PD including akinesia and bradykinesia, tremor, rigidity, gait disturbance, impaired handwriting, grip force, speech deficits, and others.[8] 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 the model organism *Caenorhabditis elegans* in aging research enables easy experimental assays because of its comparatively short lifespan of just ∼3 weeks.[9−11] As *C. elegans*

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ages, it displays many readily observable and quantifiable changes such as tissue degeneration, decreased movement, and the cessation of reproduction.[6,12,13] More importantly, monitoring age-related diseases is also made easy since more than 83% of the genes are similar to human beings including those that are related to neurological diseases.[14] *C. elegans* has a well-defined nervous system composed of exactly 302 neurons. Unlike any other organism, the connections of these 302 neurons in *C. elegans* have been completely mapped, thereby providing the most complete nervous system connectome of any organism.[15] These neurons are responsible for complex behaviors including chemotaxis, thermotaxis, touch response, mating rituals, social and individual feeding, and scavenging as well as associative and nonassociative learning.[15−24]

There have been *C. elegans* mutant strains for Parkinson's disease study such as BZ555, of which GFP fluorophores are attached to the dopamine transporter DAT-1 on the dopaminergic neurons;[25−28] NL5901 that expresses human α-synuclein attached with a yellow fluorescence protein (YFP);[27,29,30] and worms with a mutation in dopamine synthesis genes such as *cat-2, cat-4*, and *bas-1*.[24,28,31,32] These worms exhibit Parkinson's disease-like symptoms including degeneration of dopamine neurons, loss of dopamine-related behaviors, deterioration in movements, and aggregation of α-synuclein.[33,34]

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

2. RESULTS

2.1. DAF-2-Independent Regulation of the Lifespan by a High-Glucose Diet. In previous studies, wild-type *C. elegans* (N2) grown on a medium containing high concentrations of glucose showed a shortened lifespan.[35−39] In this study, the high concentration (250 mM) of glucose was toxic to the lifespan of wild-type *C. elegans*, ∼50% of animals were dead at 8 days after adulthood, and shortened the lifespan of this strain of mutation by 15% in DAF-2 (Figure 1). All of the worms fed with high glucose were dead before 15 days of adulthood, while some animals without glucose feeding lived for 20 days in adulthood. In comparison with our previous study,[37] the survival ratio of animals grown with 250 mM glucose is much smaller than that of worms fed with 10−20 mg/L glucose, which demonstrates that the toxicity of a glucose diet is exerted in a dose-dependent manner.

The insulin/IGF-1 signaling pathway and its connection with aging have been established in *C. elegans* as well as in many other organisms. The daf-2 is a key regulator gene encoding an insulin-like receptor that intervenes in endocrine signaling. The DAF-2 protein has been reported to be 35% identical to the human insulin receptor, 34% identical to the human insulin-like growth factor-I (IGF-I) receptor, and 33% identical to the human insulin receptor-related receptor.[36−38] The reduction of daf-2 signaling in adulthood causes lipid accumulation and an extended (approximately double) lifespan.[35] In the present study, as expected, the mutant strain *daf-2* (*e1370*) lived longer than the wild-type N2 strain in the case of no glucose diets; however, the high-glucose diet shortened the lifespan of this strain of mutation in DAF-2 (Figure 1).

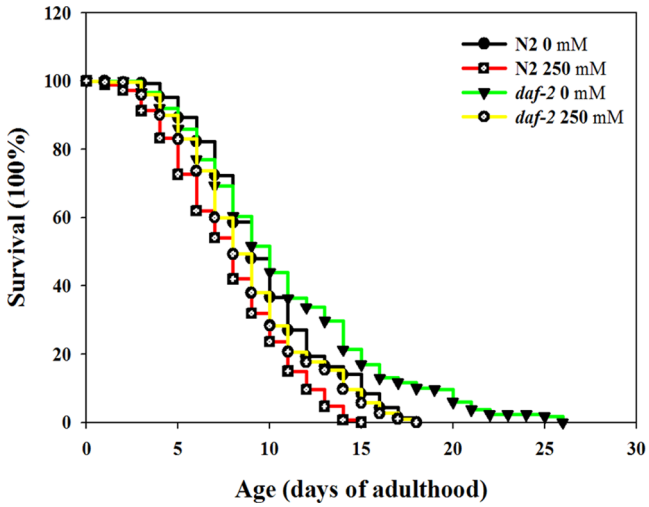

Figure 1. High-glucose diet shortens lifespan of *C. elegans*. Both wild-type N2 and daf-2 mutant worms' lifespans were significantly shortened by 250 mM glucose consumption. All trials were done three times, and each trial has $N \geq 100$ worms per group. The statistical analysis of the lifespan was performed by Kaplan−Meier survival analysis for each group.

Given that the lifespan of *C. elegans* is governed by the genetic regulatory pathway with DAF-2 in the upstream,[35] the high-glucose-diet-induced short lifespan in *daf-2* (*e1370*) suggests a DAF-2-independent regulation of the 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 a difference in the survival ratio.

2.2. Deterioration of Body Size in Early Adulthood by a High-Glucose Diet. *C. elegans*' body size has been used as an indicator of the progress of aging or biological toxicity. Endogenous or exogenous conditions to accelerate the aging of *C. elegans* led to an increase in body size, while the uptake of toxic compounds resulted in a decrease in body size or suppression of body growth.[40,41] 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 (Figure 2). The body sizes of N2, CB1370, and NL5901 strains were 10, 32, and 32% longer than those of worms without a 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 Figure S1). Considering that the increase in body size was enhanced in *daf-2* mutant worms, the high-glucose-diet-induced acceleration of aging progress might be partially downregulated by DAF-2. The high-glucose diet showed a synergetic effect in the 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 the 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 the high-glucose diet on dopaminergic neuronal behaviors.

2.3. Slowness of the Body Movement during a High-Glucose Diet. Slowness of the body movement is one of the representative behaviors accompanying aging and Parkinson's disease,[42] which was observed in the worms fed with the high-

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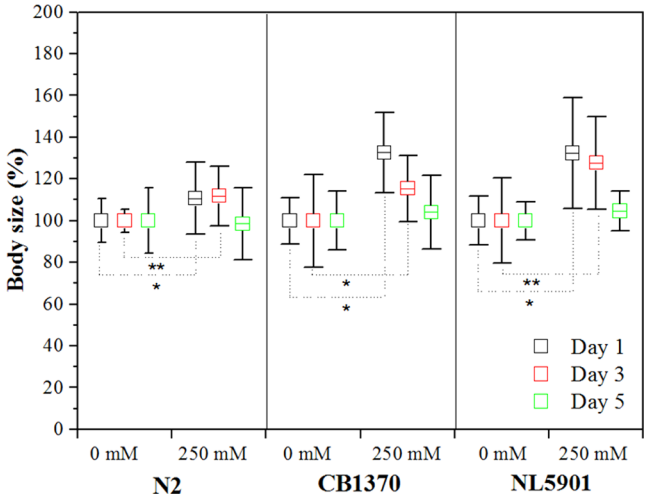

Figure 2. High-glucose diet increases the body size of *C. elegans*. Body lengths of wild-type N2, CB1370 (daf-2), and NL5901 (human α-synuclein::YFP) were significantly enlarged by 250 mM glucose consumption from 1 day of adulthood. Measurement of the worm's body size was done until day 5 of adulthood. At least three trials were conducted with $N \geq 100$ worms per group (Student's *t*-test: $^{*}p < 0.05$, $^{**}p < 0.01$).

glucose diet. Although the survival ratio of worms fed with high-glucose diets was similar to control animals, the slowness of the body movement was significantly induced by 250 mM glucose in early adulthood (day 3) (Figure 3). The wild-type

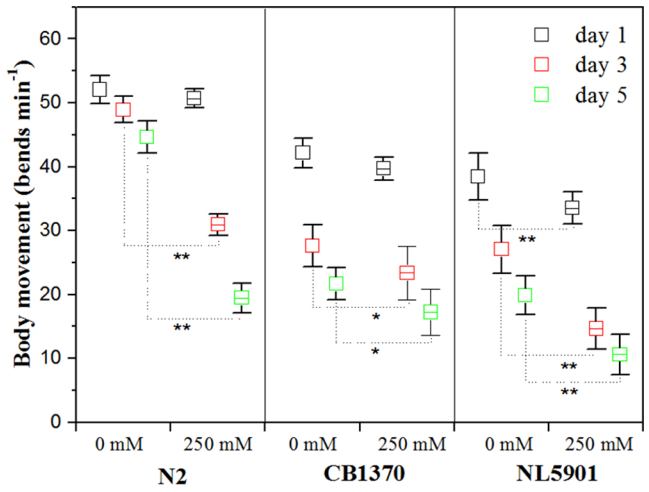

Figure 3. High-glucose diet deteriorates the body movement of *C. elegans*. Body movements of wild-type N2, CB1370 (daf-2), and NL5901 (human α-synuclein::YFP) were significantly diminished by 250 mM glucose consumption from 1 day of adulthood. Body bends were recorded by counting only spontaneous forward oscillations (Video S1), and the movement assay was done until day 5 of adulthood. At least three trials were conducted with $N \geq 100$ worms per group (Student's *t*-test: $^{*}p < 0.05$, $^{**}p < 0.01$).

N2 worms without the high-glucose diet showed an 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 a high-glucose-diet-induced movement slowness was also found in the *daf-2* mutant strain (CB1370) during the same period of adulthood, which implies that the shortened lifespan and acceleration of aging behavior by 250 mM glucose is independent of the DAF-2 regulatory pathway. The high-glucose diet drastically accelerated the progress of the body movement slowness in the NL5901 strain expressing human α-synuclein from day 1 in adulthood. These results suggest that the DAF-2-independent lifespan dysregulation might be highly associated with dopaminergic neuron degeneration and the genetic regulatory pathway for Parkinson's disease.

2.4. Degeneration of Dopaminergic Neurons by a High-Glucose Diet. To identify whether the slowness of the body movement in the animals fed with a high concentration of glucose was linked with the degeneration of dopaminergic neurons; the fluorescence from the Pdat-1::gap strain (BZ555) was examined under a microscope. The DAT-1 gene is specifically expressed in the dopaminergic neurons and is involved in the transportation of dopamine between neuronal cells.[43] Before the onset of the body movement slowness by a high-glucose diet, the dopaminergic neurons of *C. elegans* fed with 250 mM glucose were significantly inactivated or degenerated with the significant reduction of the Pdat-1::gfp fluorescence intensity at 12, 24, and 48 h of adulthood (Figure 4A). Quantitative analyses using the ImageJ computer program revealed that dopaminergic neurons of worms fed with 250 mM glucose showed a 20−40% loss of fluorescence intensity, suggesting the inactivation or degeneration of dopaminergic neurons (Figure 4B).

The anterior part or the head of *C. elegans* contains two types of dopaminergic neurons, ADE and CEP.[43] To identify which neuron was predominantly degenerated by a high-glucose diet, we observed the fluorescence of each type of dopaminergic neuron by observing both ADE and CEP independently (Figure 5). In particular, the fluorescence level of the ADE neuron was dominantly diminished with an increase in glucose concentrations, suggesting that a high-glucose diet led to the degeneration of the ADE neuron. Quantitative analyses using the 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 Figure S2). The results suggest that ADE neurons are more susceptible to a high-glucose diet than CEP neurons in dopaminergic neuronal systems in *C. elegans*.

To verify whether the reduction of fluorescence intensity demonstrates either damage to dopaminergic neurons or the downregulation of the dat-1 promoter, we observed the morphology of dopaminergic neurons located in the anterior part of *C. elegans*. Compared with the control group (without a high-glucose diet), the dopaminergic neurons of *C. elegans* fed with a high concentration of glucose showed a defective morphology, including a not-solid and disconnected line shape of neurons (see Figure S3). The defectiveness originating from high glucose was much worse than that from 6-OHDA, known as a drug inducing neuron damage (see Figure S3). These results demonstrated that the reduction or loss of fluorescence intensity might be due to the morphological damages or defectiveness in dopaminergic neurons rather than the downregulation of the dat-1 promoter.

2.5. 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* is placed on an agar plate in the presence of bacterial food, the

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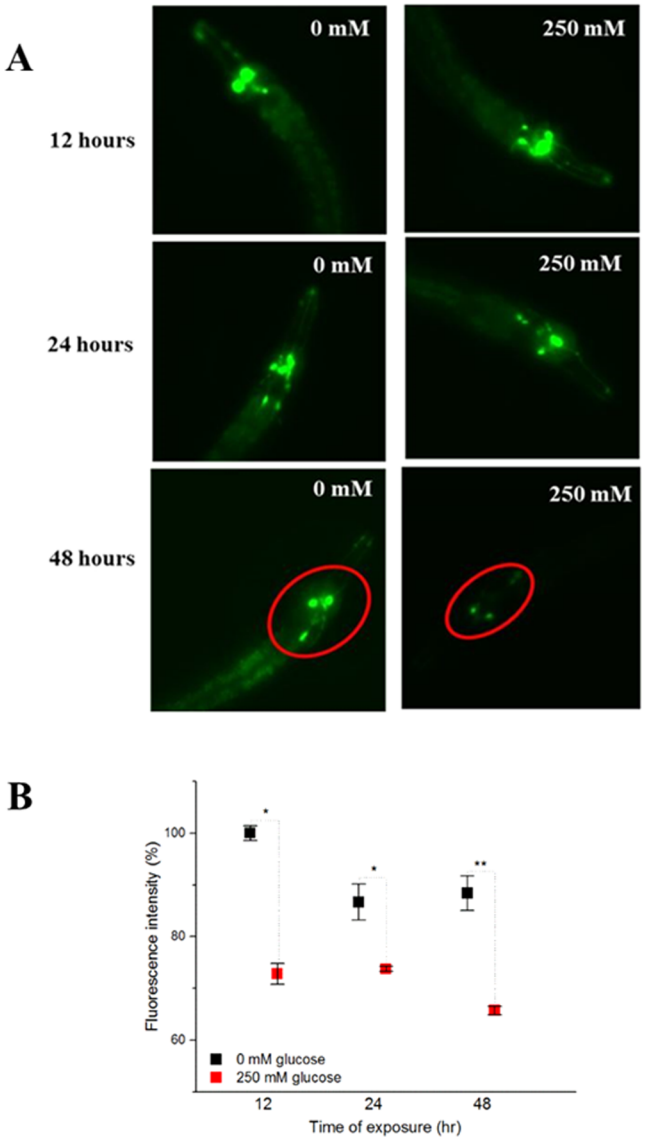

Figure 4. High-glucose diet inactivates dopaminergic neurons of *C. elegans*. Fluorescence intensities of Pdat-1::gfp expressed in dopaminergic neurons were significantly reduced by 250 mM glucose consumption from 12 h of exposure in a time-dependent manner (A, B).

animals show slower crawling locomotion compared with those in the absence of bacterial food.[42] 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 a high-glucose diet showed slower locomotion when placed on the (+) food plates compared with worms on the (−) food plates (% BSR: 60.98, Figure 6A) (Videos S2 and S3). However, high-glucose (50, 100, and 250 mM) diets led to a decrease in BSR percentages with values of 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 the case of worms fed with high concentrations of glucose (Figure 6A). These results indicate that the high-glucose-diet-induced deterioration of the dopaminergic neuronal system, dopamine synthesis, and transport disables worms' responsible locomotion.

Since the BSR behavior is governed by the dopaminergic neuronal system, the BSR is significantly diminished when dopamine is hardly produced by the mutation in CAT-2, a tyrosine hydroxylase, an enzyme necessary for dopamine synthesis in *C. elegans*.[43] The BSR percentage significantly decreased to 12.6% in *cat-2* (e1112) mutant worms from 61.0% (wild-type N2) without a high-glucose diet (Figure 6B) (Videos S4 and S5). The percentage of BSR in *cat-2* (e1112) reduced from 12.6 to 4.8 and 6.4% in the worms fed with 100 and 250 mM glucose, respectively (Figure 6B). 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 Pdat-1::gfp, indicating the inactivation of dopamine transport in *C. elegans* fed with high concentrations of glucose.

2.6. Decrease in *In Vivo* Dopamine Levels by a High-Glucose Diet. Wild-type N2 worms with a high-glucose diet showed *cat-2* mutant-like, dopamine-depletion behaviors. To verify whether the dopamine content decreased in *C. elegans* with a high-glucose diet, the quantitative measurement of the *in vivo* dopamine level was performed by HPLC analysis using whole-body extracts from worms at 1 day of adulthood. The dopamine level of high-glucose diet N2 worms was about 15 pg/mg protein, which was 35% of the dopamine content in the control N2 group (42 pg/mg protein, without the glucose diet) (Figure 7). This result indicates that 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 (Figure 4A). However, the dopamine contents were not significantly different between the control group (0 mM glucose) and the high-glucose diet group (250 mM glucose) in Pdat-1::gfp strains BZ555 (Figure 7). These results demonstrate that high-glucose diets degenerate dopaminergic neuronal systems by reducing dopamine transport through DAT-1 despite the same or similar dopamine levels in the body.

2.7. Aggregation of α-Synuclein by a High-Glucose Diet. In the human Parkinson's disease model, α-synuclein, a presynaptic cytoplasmic protein is reported to regulate dopamine transport.[44] However, the aggregation of α-synuclein to form Lewy bodies is highly linked to the progress of Parkinson's disease by the dysregulation of the dopaminergic system. To investigate the effect of a high-glucose diet on α-synuclein aggregation, NL5901 strain worms,[29,45,46] in which human α-synuclein fused with a yellow fluorescent protein (YFP) is expressed in the body wall muscle, were observed under a fluorescence microscope. When the glucose concentration increased from 50 to 250 mM, the number of visible α-synuclein aggregates increased from 20 to 60 in a dose-dependent manner (Figure 8). Considering *C. elegans* as a human surrogate system, this result demonstrates that the high-glucose diet has a possibility to cause the dysregulation of dopamine synthesis and transport through abnormal and enhanced α-synuclein aggregation.

3. DISCUSSION

The linkage of several neurodegenerative diseases including Parkinson's disease with type 2 diabetes is one of the most discussed research.[47−49] Previous studies have shown that an

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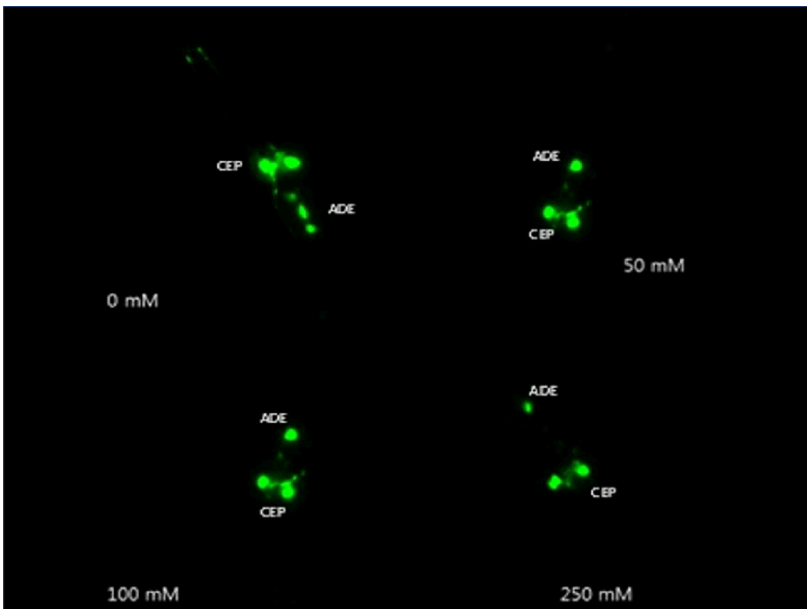

Figure 5. High-glucose diet induces glucose concentration-dependent and neuronal cell-specific degeneration of the dopaminergic neurons in *C. elegans*. Worms were exposed to different concentrations of glucose ranging from 50 to 250 mM for 12 h to observe the degradation of dopaminergic neurons. Among dopaminergic neurons, ADE neurons were more dominantly degenerated by a high-glucose diet.

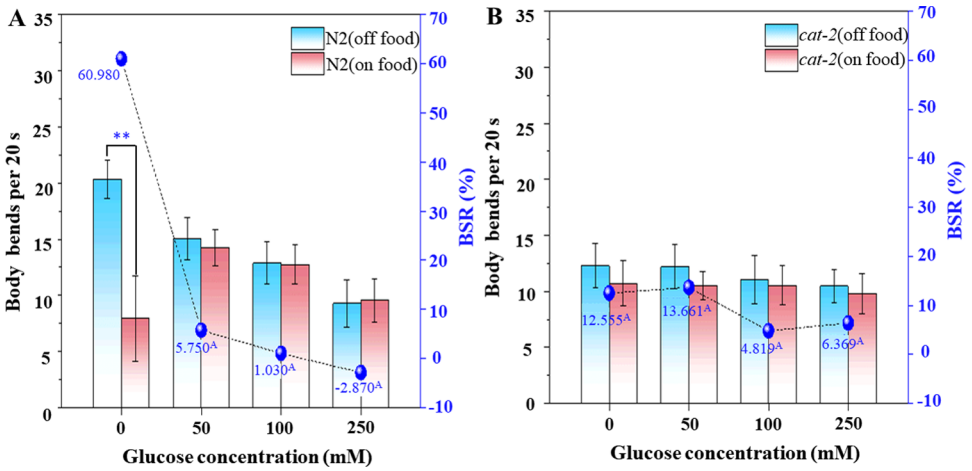

Figure 6. High-glucose diet deteriorates dopaminergic behavior and basal slowing response (BSR). Wild-type N2 worms exhibited a loss of BSR upon feeding with increasing concentration of glucose (A). The *cat-2* (*e1112*) mutant worms, on the other hand, failed to exhibit BSR, but upon further exposure to glucose, % BSR also decreased (B). The numerical data represent the mean from three independent experiments with $N = 30$. (**$p < 0.001$) ^{A} means defect in BSR compared to the wild type ($p < 0.001$).

individual's high glycemic index is more prone to developing such diseases, suggesting a high prevalence of insulin resistance in Parkinson's disease patients.[50] However, the association mechanism between Parkinson's disease and the insulin signaling pathway remains unknown up to this date. Here, we suggest that a high-glucose diet causes a DAF-2-independent regulation of the 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 the 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 a high-glucose diet on toll-like receptors and downstream regulators of insulin and the IGF-1 signaling (IIS) pathway are required to elucidate the mechanism explaining our results.

Other studies have pointed out that the decrease in lifespan involves glycolysis-related enzymes.[51] In a separate study conducted by Lee and his group, a step-by-step inhibition of the glycolytic pathways resulted in discovering that glucose GPI or fructose-1,6-biphosphate aldolase (ALDO 1,2) inhibition was a great tool to suppress glucose toxicity.[52] On an additional note, methylglyoxal is inevitably formed as a byproduct of glycolysis. In an *in vitro* experiment by van Hinsbergh and his group, endothelial cells incubated in 30 mM D-glucose promoted an estimated 2-fold higher production of methylglyoxal. Methylglyoxal is a highly reactive dicarbonyl compound that is a major cell-permeant precursor of advanced glycation end products (AGEs). This glycolysis byproduct is known to be associated with several pathologies, including

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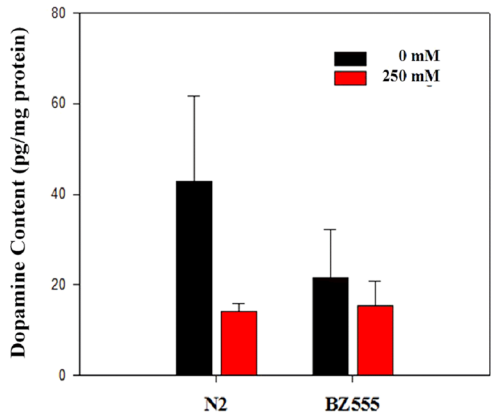

Figure 7. High-glucose diet reduces the dopamine synthesis level of *C. elegans*. Total dopamine contents measured using HPLC analysis were significantly reduced in wild-type N2 and slightly in BZ555 (Pdat-1::gfp) by 250 mM glucose consumption. The numerical data represent the mean from three independent experiments with $N > 3000$ per sample ($*p < 0.01$).

diabetes, aging, and neurodegenerative diseases.[53] Bae and his colleagues reported that methylglyoxal plays a part in the cerebrovascular complications development in diabetic patients and pointed out the roles of oxidative stress and mitophagy in MG-induced functional damage in brain endothelial cells (ECs).[54] Some reports brought up an increase in the cellular reactive oxygen species (ROS) levels, which was responsible for the dysregulation of cellular signaling and a variety of genetic pathways, thereby causing apoptosis and cell death.[55,56] In the separate study that we previously reported, the lifespan of *C. elegans* was shortened via ectopic apoptosis.[37] With additional pieces of evidence, as age progresses, glucose metabolism shifts from aerobic to anaerobic, and persistent glycolysis results in the increase of ROS generation, which provokes negative effects on the mitochondria.[57]

In a recent study, Pinkas et al. discussed that a 400 mM glucose solution resulted in a significant decrease in the fluorescent signal in the dopaminergic neurons (around 40% dopamine reduction, from 60.56 to 36.54, $p < 0.01$), cholinergic system, (around 53% reduction in acetylcholine, from 58.5 to 27.72, $p < 0.0001$), and glutamatergic system (around 42% glutamate reduction, from 39.95 to 23.12, $p < 0.0001$).[58] While the Pinkas group used 14-day-old worms, our study investigated the effect of a high-glucose diet on the dopaminergic neurons at a very early age, 12, 24, and 48 h from L4 stages, which is equivalent to young adult, day 1, and day 2 adult worms, respectively. Based on the current observation, 80% of DAT-1 was inactivated in the worms fed with 250 mM glucose at 12 h of adulthood.

Among the dopaminergic neurons in *C. elegans*, ADE neurons were particularly inactivated with less fluorescence intensity from Pdat-1::gfp than in the worms exposed to high concentrations of glucose in comparison with CEP neurons. Given that CEP neurons are responsible for food recognition, the stronger fluorescence intensity of CEP neurons indicates that the defectiveness in basal slowing response (BSR) behavior was caused by a low dopamine level or transport of dopamine, not by food recognition (Figure 6). Unlike CEP neurons, ADE neurons contain tyramine receptors in addition to dopamine receptors, and tyramine is a precursor or alternative to 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. More deterioration of ADE than CEP was also found in the previous study[59] focusing on a synergetic effect of a glucose-rich diet on insecticide-induced dopaminergic neuronal dysfunction in *C. elegans*. Without insecticides, we here found that a 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 in Parkinson's disease-linked kinase activity-dependent manner.[60] Given that the glucose-rich diet specifically degenerated ADE neurons containing the D2 class dopamine receptor,[61] 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 a glucose-rich diet (Figure

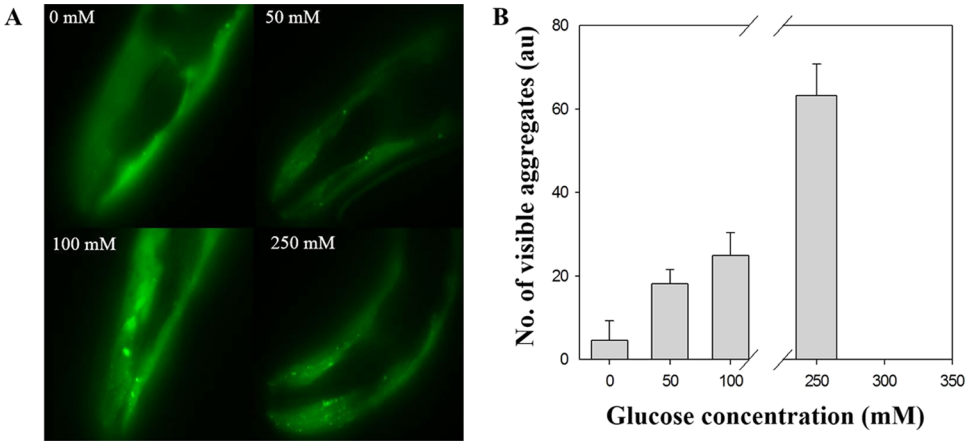

Figure 8. High-glucose diet induces and increases α-synuclein aggregation in muscles of *C. elegans*. A close-up image of the α-synuclein aggregates in a transgenic worm (A) and the quantification of visible α-synuclein aggregates (B). The number of α-synuclein aggregates was drastically increased in day 1 old NL5901 worms expressing human α-synuclein in a dietary glucose concentration-dependent manner. $N \geq 100$; three replicates.

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8) in comparison with the 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*.

In conclusion, a high-glucose diet accelerates aging progress along with body size increase and body movement decrease in *C. elegans* in a *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. Thus, the control of glucose consumption has the potential for slowing aging and neurodegenerative disease progression.

4. MATERIALS AND METHODS

4.1. 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 [Pdat-1::gfp]], and CB1112 [cat-2 (e112)] were obtained from the Caenorhabditis Genetics Center (CGC, University of Minnesota, Twin Cities, MN). Standard conditions were used for *C. elegans* propagation at 20 °C, while CB1370 [daf-2(e1370)] worm strains were propagated at 25 °C. Worms were synchronized by hypochlorite bleaching, hatched overnight, and subsequently cultured on NGM plates with OP50. The synchronized worms were prepared and grown until the L4 larval stage.

4.2. Method Details. *4.2.1. Analysis of Lifespan.* Lifespan analysis of *C. elegans* was conducted following a previously described method with slight modification.[62] The test plates were prepared by adding 250 mM glucose to NGM plates before seeding with *Escherichia coli* OP50. The optimization of the glucose concentration was determined by the concentration showing dramatical toxicity in the survival ratio among the five different concentrations (0, 10, 100, 250, and 500 mM). Lifespan was scored by transferring control worms and glucose-treated worms to new control or glucose-containing plates, respectively. Nematodes were transferred every day during the reproduction stage and every other 3 days after the reproduction stage until all worms were dead. Worms that were not moving were counted as dead, while worms that crawled out of the plates were counted as missing. All trials were done three times, and each trial had $N \geq 100$ worms per group. The statistical analysis of lifespan was performed by Kaplan−Meier survival analysis for each group.

4.2.2. Body Movement Assay. Overall, 10 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. The bends were recorded by counting only spontaneous forward oscillations (Video S1). Bends were counted for 60 s. Locomotion assay was done until day 5 of adulthood. At least three trials were conducted with $N \geq 100$ worms per group.

4.2.3. C. elegans Body Size Assay. Overall, 10 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 a digital camera (C-5050 zoom, Olympus, Japan). The body size of the individual worm was analyzed 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 $N \geq 100$ worms per group ($^*p < 0.05$, $^{**}p < 0.01$).

4.2.4. Dopaminergic Neuron Observation. A study of dopaminergic neurodegeneration was carried out by exposing the synchronized L4 BZ555 worms to 250 mM glucose at different time intervals of 12, 24, and 48 h. After treatment, nematodes were washed with an M9 buffer to remove adhering bacteria on the bodies, and then worms were transferred to 2% agarose pads on glass slides mounted with 5 M Levamisole and enclosed with a coverslip. Imaging of living (immobilized) worms was carried out to monitor the neurodegeneration by observing the green fluorescence (GFP) attached to the dopamine neurons using a 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 in three trials in each set. In a separate set of experiments, nematodes were treated with increasing concentrations of glucose (50, 100, and 250 mM) for 12 h. Again, after treatment, worms were mounted to an agarose pad as described above and were observed using the Axio Imager A2 and analyzed using ImageJ software.

4.2.5. Basal Slowing Response. Basal slowing assays were done using a previously described (Chase et al.[42]) with a 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 min. After 5 min, half of the worms were then transferred to the "with-food plate" (NGM media with seeded OP50) and the other half to the "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 s was determined for each condition. Data were 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.

4.2.6. Quantitative Analysis of α-Synuclein Accumulation. Accumulation of α-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 20 °C for 24 h. After treatment, 10 randomly selected day 1 old nematodes were then transferred to 2% agarose pads on glass slides, mounted with 5 M Levamisole, and enclosed with a coverslip. Immobilized worms were observed and imaged using an 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 aggregation of proteins in the NL5901 strains was scored by counting the number of visible spherical-shaped α-synuclein aggregates individually. The assay was done in three replicates.

4.3. 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 the Student *t*-test (SigmaPlot 10.0, SPSS Inc.,

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Chicago, IL). 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 $^{***}p < 0.001$).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03384.

Body sizes of all strains upon 250 mM glucose feeding, glucose concentration-dependent and neuronal cell-specific dopaminergic neuron degeneration, and dopaminergic neurons in different diets (PDF)

Video S1: body movement recording (MPG)

Video S2: basal slowing response of control N2 worms (not treated with glucose diet; "nonfood" plate) (MPG)

Video S3: basal slowing response of control N2 worms (not treated with glucose diet; "on-food" plate) (MPG)

Video S4: basal slowing response of glucose-treated N2 worms (treated with glucose diet; "nonfood" plate) (MPG)

Video S5: basal slowing response of glucose-treated N2 worms (treated with glucose diet; "on-food" plate) (MPG)

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Author Contributions

A.C.V.d.G. and S.S.C. designed the experiments. A.C.V.d.G. performed *C. elegans* cultures and assays and took all supporting videos. E.J.K. performed the HPLC analysis. S.K. performed the qPCR experiments. J.H.C. and J.H.K. guided A.C.V.d.G. for microscopic analysis. S.S.C. and A.C.V.d.G. wrote the manuscript. All authors reviewed and contributed to the manuscript.

Notes

The authors declare no competing financial interest.

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