The Impact of Artificial Sweeteners and Natural Sweeteners on The Lipid and Carbohydrate Metabolism of *Caenorhabditis Elegans*

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Abstract. There has been ongoing controversy in the scientific community regarding the safety and health effects of artificial sweeteners and natural sweeteners. To explore the potential safety hazards of sweeteners on the human body, this study used the model organism *Caenorhabditis elegans* (C.elegans) and inoculated them in solutions of glucose, the artificial sweetener aspartame, and the natural sweetener stevioside. The experiment observed and recorded the worm's egg-laying number, pumping number/30s, relative glucose levels, and relative lipid content. Additionally, transcriptome sequencing was performed on the C. elegans samples from the 3 mg/ml aspartame group, 10 mM stevioside group, 100 mM glucose group, and control group, using the wild-type strain N2 as the reference genome. Gene expression levels were calculated using the TPM method, and differentially expressed genes were identified using the FDR < 0.05 threshold. Pathway enrichment analysis of the differentially expressed genes was conducted using the KEGG and Gene Ontology (GO) Biological Process (BP) databases. Integrating the physiological and biochemical indicators with the analysis of differentially expressed genes, it was found that aspartame may increase relative glucose levels and reduce relative lipid level in the body by upregulating *precursor energy metabolism* and *oxidative phosphorylation* pathways. Conversely, the experiment suggests that stevioside may lower relative glucose levels and promote relative lipid level by downregulating catabolism and lipid metabolism. This indicates that caution should also be exercised when consuming stevioside, which is considered a relatively healthy natural sweetener. Furthermore, this study provides new research directions for understanding the effects of artificial sweeteners and natural sweeteners on organism metabolism.

Keywords: *Caenorhabditis elegans*, sweeteners, aspartame, stevioside, lipid metabolism, carbohydrate metabolism.

1. Introduction

Sweeteners are chemical substances widely used to replace traditional sugars to enhance food and beverages sweetness and reduce sugar intake. Sweeteners can be broadly classified into two categories: artificial sweeteners such as aspartame (AP) and natural sweeteners such as stevioside (SV). [1]

United states in 2016, and European nation in 2006 approved the safety of sweeteners usage[2], [3]. Also, numerous studies indicate that replacing traditional sucrose with sweeteners can decrease body weight, insulin resistance, body fat, and blood glucose in human[1]. However, the health impact of sweeteners has been controversial. In 2023, the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) classified AP as “possibly carcinogenic to humans,” which spikes global attention towards the safety of sweeteners[4].

Lipid and carbohydrate metabolism are highly conserved processes that nearly impact all aspects of an organisms’ biology. Abnormal human glucose and lipid metabolism serves as indicator for chronic diseases including diabetes, hypertension, and hyperlipidemia[5]. In previous researches, sweeteners have been found to be associated with lipid and carbohydrate metabolic disorder. For instance, a systematic review and meta-analysis based on prospective cohort studies in healthy individuals have shown a positive correlation between artificial sweetener intake and the incidence of type 2 diabetes mellitus[1]. Also, many other epidemiological studies show that consumption of sweeteners can lead to cardiovascular disease, weight gain, hyperlipidemia, and hypertension, but the

mechanism of sweeteners influence on lipid and carbohydrate metabolism is unclear[1]. A human study showed that AP has a lipid-mediating effect by increasing gene expression of SOD1/2, PINK1, and FIS1[6]. In mammals' experiment, the consumption of AP can dysregulate blood glucose and blood lipids by upregulate lipopolysaccharide synthesis related genes[7]. The *Caenorhabditis elegans* (*C. elegans*) experiments have analyzed the effects of different sweeteners on some specific genes related to insulin, carbohydrate metabolism, and lipid metabolism, such as daf-2, daf16, IGF-1, aak-2, lip-1, and gck-1. The results indicate that sweeteners may have certain effects on sugar and lipid metabolism, as well as nucleotide metabolic pathways in nematodes[8].

This study employed *C. elegans*, a nematode species known for its highly conserved metabolic pathways shared with humans [9], to investigate the effects of artificial and natural sweeteners on various *C. elegans'* physiological aspects. Furthermore, RNA sequencing analysis was conducted to identify differentially expressed genes, providing insights into molecular-level changes in glucose and lipid-metabolism.

2. Method

2.1. Nematode maintenance and synchronization

The *C. elegans* L4 stage larvae were cultured on Nematode Growth Media agar plates in a 20°C incubator. To achieve synchronous development of L4 stage *C. elegans*, the nematodes were transferred from NGM plates onto fresh culture dishes. Each individual dish has 20 *C. elegans* specimens. The L4 stage *C. elegans* were divided into 16 groups, with each group consisting of 20 nematodes. Different concentrations of glucose, AP, and SV solutions were added to each group. Table 1-1 shows 16 treatment groups.

Table 1-1 Groups of different drug's treatment.

Group	**1**	**2**	**3**	**4**	**5**
Glucose (mM)	10	20	40	80	100
Stevioside (mM)	0.1	0.3	1	3	10
Aspartame (mg/mL)	0.03	0.1	0.3	1	3
Control	0	0	0	0	0

2.2. Egg laying assessment

The number of eggs laid by the *C. elegans* was recorded over a 24-hour period after the treatment, with three repetitions performed.

2.3. Pumping number/30s assessment

Each group of *C. elegans* was observed under a microscope (BSW-CS-5, Shanghai Qibu Biotechnology Co.,Ltd) with a magnification of 40-50x, and the frequency of pharyngeal contractions was recorded for a 30-second duration.

2.4. Measurement of glucose concentration

The glucose concentration was determined with a BCA assay kit (Vazyme Biotech Co.,Ltd, E112-01) after 7 days of incubation on NGM plates containing different concentrations of glucose, AP, and SV at 20°C. The absorbance was measured at 630 nm. The glucose levels of the experimental groups were compared to those of the control group, and the relative values were calculated.

2.5. Nile red staining and analysis

The D4 nematodes were fixed in a 4% paraformaldehyde (PFA) solution at a concentration of 10 minutes for a Nile Red staining assay [10] [10]and DAPI staining [11]. Nematodes were placed over the coverslips. Images were captured using a fluorescence microscope (MCE, 7385-67-3). ImageJ software was utilized for the quantification of Nile Red intensity in the anterior intestinal cells [12].

2.6. Statistical analysis

Data was presented as mean ± SEM. Statistical significance was determined using Student's *t*-test with GraphPad version 9.0. Differences were considered significant when $p < 0.05$.

2.7. RNA isolation, amplification, library preparation, and sequencing

Total RNA extraction was performed using the standard Trizol method [13], and amplified by the Nugen Ovation® RNAseq v2 kit. The resulting cDNA was sheared to an average size of approximately 200 bp using the Covaris E220 system. For library preparation, the Illumina TruSeq DNA Sample Prep was used, with 1 μg of amplified cDNA as input.

To evaluate the performance of different amplification methods, a subset of samples was also amplified using the SMARTer Stranded Total RNA kit-pico input mammalian. No significant differences were observed between the two methods, and the samples amplified using different methods showed good clustering. The resulting sequencing libraries were then sequenced on the Illumina HiSeq 2000 platform, generating 35-200 million reads per sample (average of 107,674,388 reads), which were subsequently mapped to the *C. elegans* genome.

3. Result

3.1. AP and SV didn't affect C. *elegans*' egg laying

Carbohydrate metabolism pathways, including glycolysis and fatty acid synthesis, regulate the reproductive capacity of *C. elegans*[9]. The control group had an average of 176.7±5.6 eggs in 24 hours post-treatment. In the glucose group is 15.08% higher than control ($p=0.0020$, $p<0.05$). Treatment of AP and SV didn't cause significant differences with the control group, no matter the concentration.

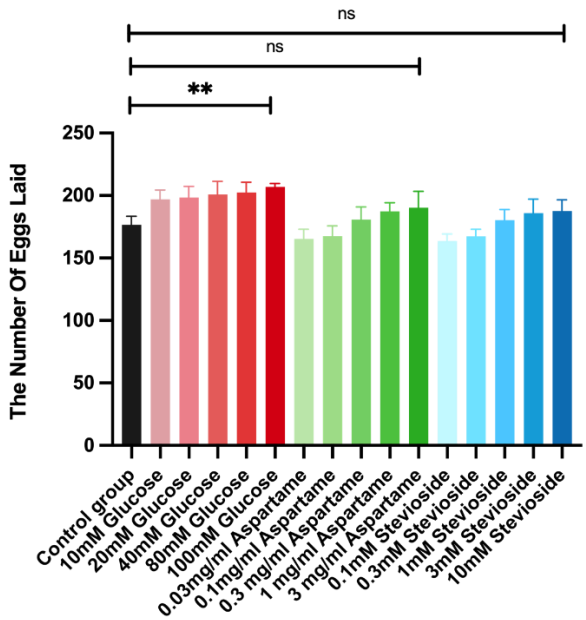

Figure 1 ｜Number of eggs laid at 24 hours post-treatment in glucose, AP and SV groups. N=20. Data is shown by Mean ± SEM; *P<0.05, **P <0.01. Student's *t*-test analysis.

3.2. Effect of AP, SV, and glucose solutions on *C. elegans* '*pumping* number/30s.

3.2.1 AP and SV decrease decline of the pumping number/30s from day 4 to day 16.

The pharynx pumping number/30s, reflecting the feeding frequency in nematodes, is a key indicator for observing energy metabolism, which plays a crucial role in overall metabolic processes [9]. Figure 2A illustrates the long-term effect of AP on the pumping number/30s of *C. elegans.*

Observations revealed a declining pumping number in the control group over time. The 100 mM glucose group exhibited less decline compared to the control group from day 4 to day 16. Similarly, the 3 mg/ml AP (Figure 2B) and 10 mM SV (Figure 2C) treatments exhibited similar trends and significant differences compared to the control group. Thus, both AP and SV, similar to glucose, decrease decline of the pumping number/30s in C. elegans.

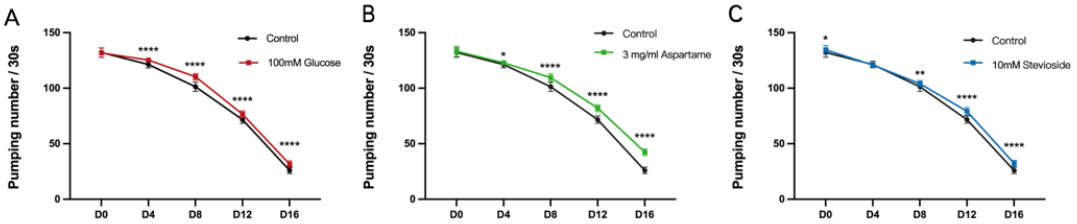

Figure 2 | Pumping number/30s of *C. elegans* during day 0, day 4, day 8, day 12, and day 16. (A) Pumping number/30s of *C. elegans* treated with 100mM glucose groups. (B) Pumping number/30s of *C. elegans* treated with 3 mg/ml aspartame. (C) Pumping number/30s of *C. elegans* treated with 10mM SV. Asterisks indicate significant difference between control and treated groups at the same day. N=30. Data is shown by Mean ± SEM. **P < 0.01, ***P < 0.001 by Student's t-test.

3.2.2 AP and SV decrease decline of the pumping number/30s dose-dependently.

To evaluate whether the effect of sweeteners on *C. elegans* pumping number/30s exhibits dose-dependency, the pumping number/30s of all groups were analyzed on day 16. Compared to the control group, the pumping number/30s showed a dose-dependent increase in the 10-100 mM glucose groups, as well as in the AP and SV groups. On day 16, the 100 mM glucose treatment resulted in a 19.23% increase in pumping number/30s compared to the control group, the 3 mg/ml AP treatment increased the pumping number/30s by 57.04%, and the 10 mM SV treatment increased it by 21.22%. Therefore, AP exhibited a greater potential to delay the decline in pumping number/30s. In general, the pumping number/30s of all three groups showed a dose-dependent increase, with the AP group exhibiting the most significant increase in pumping number/30s.

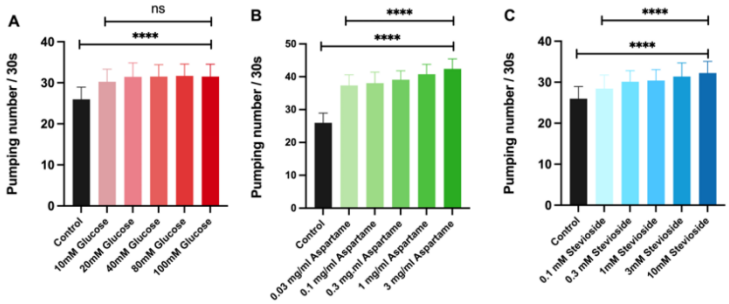

Figure 3 | Pumping number/30s of *C. elegans* in control groups and other five groups in day 16. (A) Pumping number/30s of control group and glucose groups (10, 20, 40, 80, and 100mM glucose). (B) Pumping number/30s of control group and AP groups (0.03, 0.1, 0.3, 1, and 3mg/ml AP). (C) Pumping number/30s of control group and SV groups (0.1, 0.3, 1, 3, and 10mM SV). N=30. Data is shown by Mean ± SEM. **P < 0.01, ***P < 0.001 by Student's t-test.

3.3. AP and SV effect *C. elegans* carbohydrate metabolism dose-dependently.

The relative glucose levels in *C. elegans* serve as an important indicator for assessing the nematode's carbohydrate metabolism [9]. In Figure 3, the relative glucose levels in the 100 mg/ml glucose and 3 mg/ml AP groups were 119.56% and 44.43% higher, respectively, compared to the control group ($p<0.05$). The relative glucose level in the 10 mM SV group was 45.16% lower than

the control group ($p<0.05$). Significant differences were also observed in the relative glucose levels between the 10 mM and 100 mM glucose groups, as well as between the 0.03 mg/ml and 3 mg/ml AP groups. Overall, both the AP and glucose groups exhibited a dose-dependent increase in relative glucose levels, while the SV group showed a dose-dependent decrease in relative glucose levels.

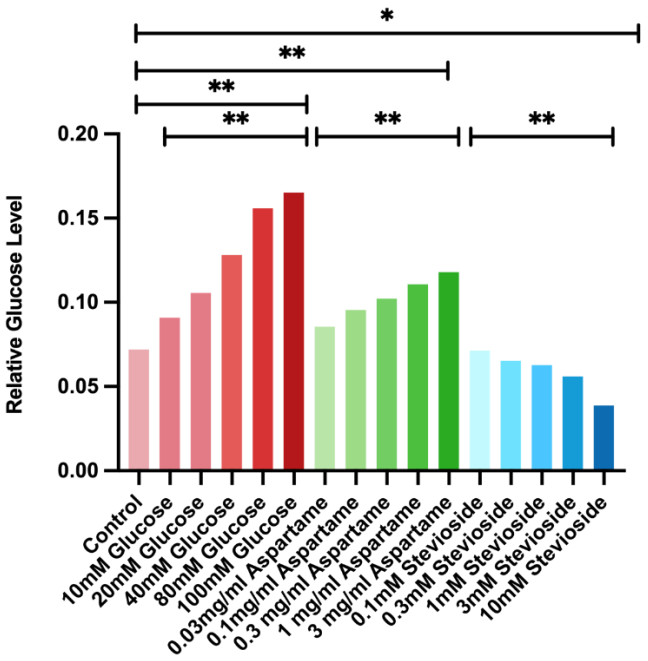

Figure 4 | glucose concentration of *C. elegans* in different groups. N=3. **Data** is shown by Mean ± SEM; *P<0.05, **P <0.01. Student's *t*-test analysis.

3.4. The effect of AP, SV, and glucose solutions on lipid deposition in *C. elegans*

To assess the effects of AP and SV on lipid deposition in *C. elegans*, we stained the lipid droplets in the nematodes' anterior intestinal cells and compared the intensity of Nile Red fluorescence after treatment with different concentrations of AP and SV solutions. Compared to the control group, the 100 mM glucose treatment group increasing the relative lipid levels by 99% (Figure 4A, 4D). Also, high doses of sweeteners significantly influenced the relative lipid levels. Particularly, 10 mM SV treatment increased the relative lipid levels by 64.73% (Figure 4C), and the 3 mg/ml AP group decreased the relative lipid levels by 61.3% (Figure 4B). Further analyzing found the effect of AP and SV on lipid deposition is dose-dependently (Figure 4E-F).

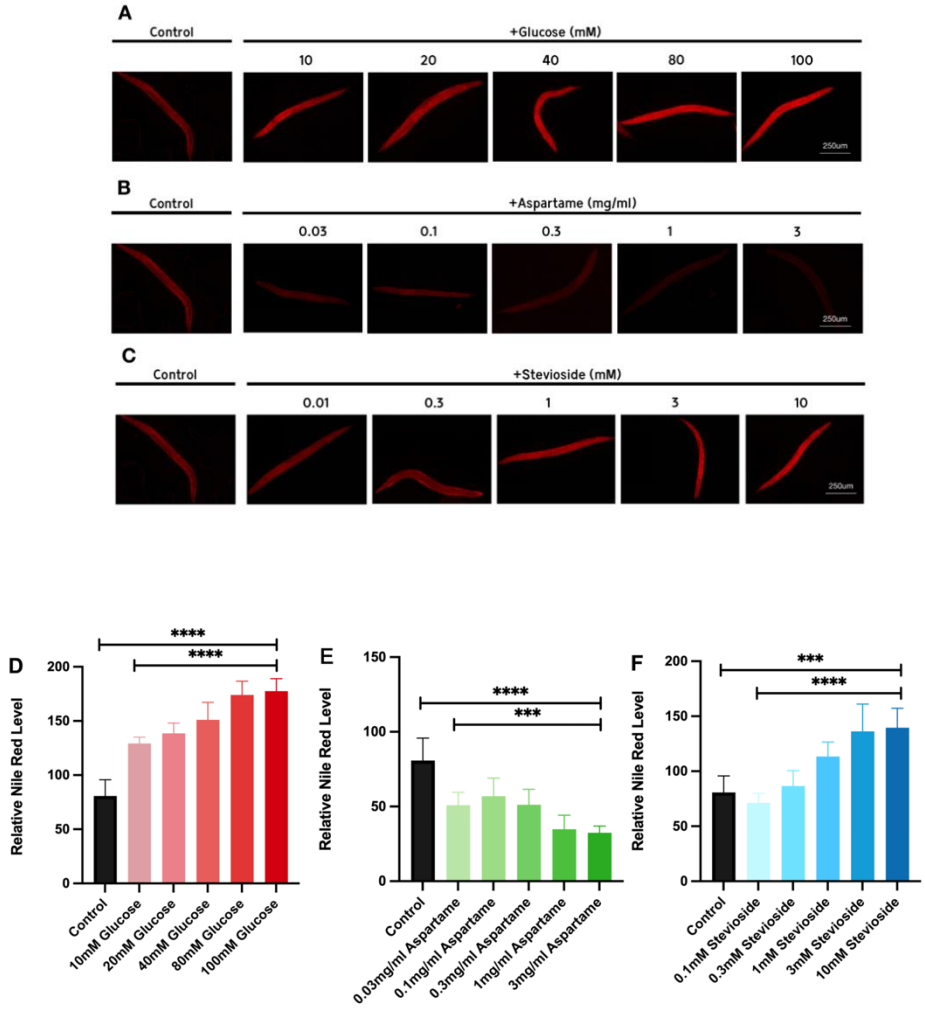

Figure 5 | Relative Nile-red level of *C. elegans* in different groups. (A-C) Nile-red staining of glucose groups (10, 20-, 40-, 80-, and 100-mM glucose), AP groups (0.03, 0.1, 0.3, 1, and 3 mg/ml AP) and SV groups (0.1, 0.3, 1-, 3-, and 10-mM SV). (D-F) The relative Nile-red level of glucose groups, AP groups and SV groups. N=6. Data is shown by Mean ± SEM. **P < 0.01, ***P < 0.001 by Student's *t*-test.

3.5. Analysis of RNA-seq results

3.5.1 Differential gene profiling uncovered the differential expressed genes in different groups

When compared to the control group (FDR < 0.05), the glucose treatment group showed 3157 differentially expressed genes, comprising 1715 upregulated genes and 1442 downregulated genes (Figure 5A). The AP group exhibited 3137 differentially expressed genes, with 1769 upregulated genes and 1368 downregulated genes (Figure 5B). In contrast, the SV group displayed the highest number of differentially expressed genes, with 6278 genes, including 3412 upregulated genes and 2866 downregulated genes (Figure 5C).

Comparing the three groups, Figure 5D reveals that the SV and glucose groups share a larger intersection, with a total of 1509 overlapping genes, compared to the intersection between AP and the glucose treatment group, which consisted of only 219 genes. This suggests a higher degree of similarity in the regulatory mechanisms of sugar and lipid metabolism pathways between SV and glucose treatments in nematodes.

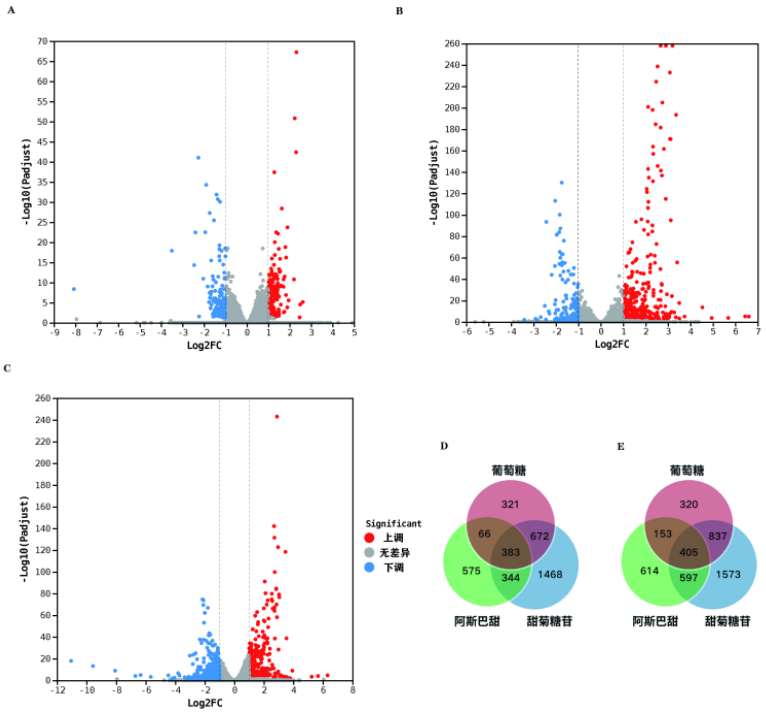

Figure 6 | Differential gene expression levels (P < 0.05) in *C. elegans* across different treatment groups. Volcano plot shows the differential gene expression between the control groups and the glucose group (A), AP group (B) and SV group (C). Venn diagram showing the overlap of differentially expressed genes among the downregulated genes in the treatment groups (D) and upregulated genes (E). In the plots, red indicates upregulated genes, blue represents downregulated genes, and gray indicates genes with no significant differential expression. Each point represents a gene, with the x-axis representing the logarithm of the fold change in gene expression between two samples, and the y-axis representing the negative logarithm of the statistical significance of the gene expression change.

3.5.2 GO and KEGG enrichment analysis (EA)

(1) 100mM Glucose treatment groups' EA

The differentially expressed genes between the 100mM glucose-treated group and the control group in *C. elegans* were mapped to the KEGG and BP (Biological Process) gene databases. Based on the enrichment results, the upregulated genes influenced the *energy acquisition and metabolism*, n*ucleic acid metabolism*, and *cellular signal transduction* in *C. elegans*. Meanwhile, *stress response*, *autophagy capacity*, *protein processing and metabolism*, *catabolic process*, and *transcriptional regulation* were downregulated.

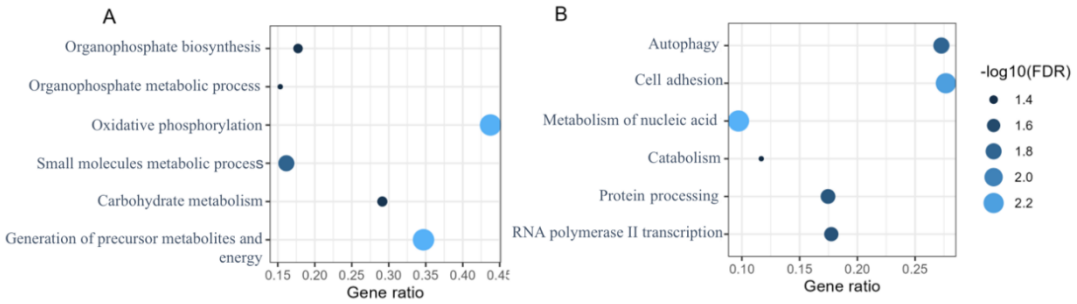

Figure 7 | GO and KEGG pathways in 100mM Glucose treatment group compared to the control group (FDR < 0.05). (A) Upregulated genes. (B) Downregulated genes.

(2) 3mg/ml AP treatment groups' EA

The enrichment results indicate that the *energy acquisition and metabolism*, *oxidative phosphorylation*, *carbohydrate and lipid metabolism*, *nucleotide synthesis*, as well as *immune and defense responses* in *C. elegans* are influenced by the upregulated genes. Meanwhile, the *self-autophagy capacity* of the nematode cells is downregulated, along with a decrease in *protein processing* and *modification capabilities*.

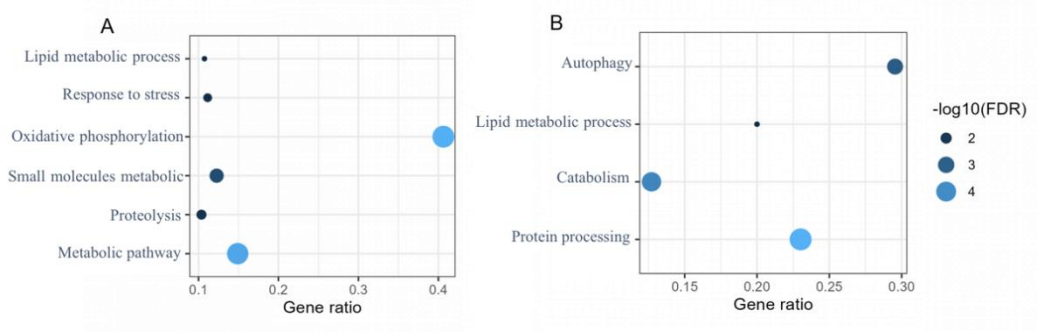

Figure 8 | GO and KEGG pathways in 3mg/ml aspartame group compared to the control group (FDR < 0.05). (A) Upregulated genes. (B) Downregulated genes.

(3) 10mM SV treatment groups' EA

According to the enrichment results, the upregulated genes influenced the *energy acquisition and metabolism*, *oxidative phosphorylation*, *nucleotide metabolism*, *nucleic acid metabolism*, *cellular localization*, and cellular autophagy in *C. elegans*. Meanwhile, *stress response*, *catabolic process*, *DNA metabolism and repair*, *metabolic pathways* (carbohydrate metabolism, lipid metabolism), and *protein processing* were downregulated.

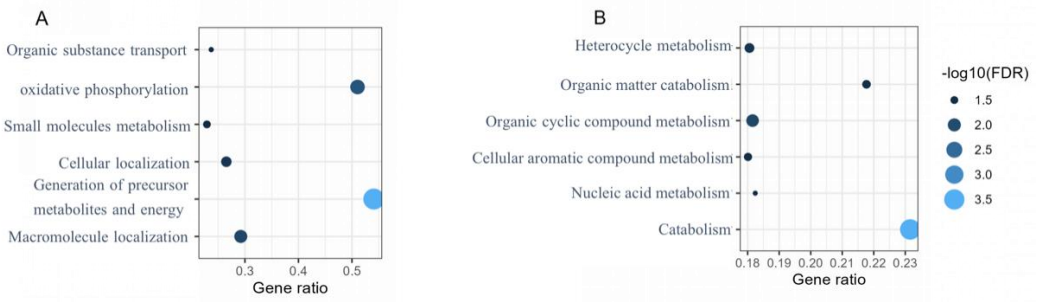

Figure 9 | GO and KEGG pathways in 10mM SV treatment group compared to the control group (FDR < 0.05). (A) Upregulated genes. (B) Downregulated genes.

Overall, the *generation and metabolism of energy* as well as *nucleotide synthesis pathways* show significant upregulation across all three groups. On the other hand, the *catabolic process* and *protein processing and metabolism* pathways are downregulated in all three groups. Comparing the three groups, both the glucose and AP groups exhibit a decrease in *cellular autophagy capacity*, while the SV group shows an increase in *cellular autophagy capacity*. Additionally, the glucose and AP groups have a higher enrichment of genes in the *oxidative phosphorylation* pathway. On the other hand, the *stress response* pathways are downregulated in the glucose and SV groups, while the *stress response and defense* mechanisms are upregulated in the AP group.

4. Discussion

Previous experiments mainly focused on exploring the impact of sweeteners on organismal physiological processes and metabolic pathways relied on observing and recording physiological indicators, phenotypic characteristics, and individual gene expression [2], [3], [5], [6]. In this study, we combined phenotypic measurements with RNA sequencing technology to assess the differential

effects of artificial sweeteners AP and natural sweeteners SV on gene expression and molecular pathways related to carbohydrate and lipid metabolism in *C. elegans.*

4.1. The impact and mechanisms of sweeteners on the egg-laying of *C. elegans.*

Studies have demonstrated the significance of normal carbohydrate and lipid metabolism in the egg-laying process of *C. elegans*, as it requires substantial energy [9]. This study found that AP and SV treatment didn't affect egg laying number, while glucose treatment increased it in *C. elegans*. The egg number results may relate to the downregulation of catabolic metabolism in AP and SV group, while glucose treatment groups did not downregulate of catabolic metabolism. In a drug test, when the catabolism of nematode went down due to injection of egl-1 drugs, the eggs laid by C. elegans are reduced [14]. Therefore, it is possible that sweeteners reduce the egg-laying number of *C. elegans* by downregulating their catabolic metabolism.

4.2. The impact and mechanisms of sweeteners on pumping number/30s of *C. elegans*

Studies suggests that pharyngeal pumping, also known as feeding behavior, is an important indicator for exploring energy metabolism in *C. elegans* [9]. The pumping number/30s of AP and SV groups declined more slowly over time compared to the control group, indicating that sweeteners can affect the feeding behavior of C. elegans. Furthermore, the gene pathway analysis illustrated that all three groups showed an increase in precursor energy generation pathways. According to studies, when the oxidative phosphorylation pathways are enhanced, the feeding frequency, or pumping rate, of nematodes is increased due to higher energy requirement [9]. Therefore, it is possible that sweeteners may increase the pumping number/30s of nematodes by enhancing *precursor energy generation*.

4.3. The impact and mechanisms of sweeteners on the relative glucose levels in *C. elegans.*

The regulation of relative glucose levels in *C. elegans* involves processes such as glucose uptake, utilization, and storage [15]. Consistent with previous research, the glucose group in this study demonstrated a dose-dependent increase in relative glucose content in *C. elegans* because glucose promotes energy generation, leading to enhanced carbohydrate metabolism and elevated relative glucose levels in *C. elegans*. Interestingly, the AP group also exhibited a dose-dependent increase in relative glucose levels, suggesting that AP may contribute to glucose elevation by enhancing energy generation. Studies conducted in mice have indicated that upregulation of the *oxidative phosphorylation* pathway enhances energy production, resulting in higher glucose demand and increased glucose intake or conversion [16], [17]. This study observed a significant upregulation of the *oxidative phosphorylation* pathway in the AP group, providing further evidence that AP may enhance glucose generation through the upregulation of this pathway.

On the contrary, as the concentration of SV increased, the relative glucose levels in nematodes decreased. Several mammals' experiments have demonstrated that lower blood glucose levels are resulted from insulin-induced reduction in catabolism [18], [19]. Specifically, when the mice's' insulin pathway being activate, the conversion of glucose to glycogen increase, and the catabolism of glycogen to glucose is prevented. In this study, the gene results shows that pathways such as *catabolic metabolism*, *organic substance catabolic metabolism*, and *organic cyclic compound metabolism* were downregulated after SV treatment. Thus, it can be inferred that the downregulation of catabolic metabolism may contribute to the decrease in relative glucose levels in *C. elegans.*

4.4. The impact and mechanism of sweeteners on lipid deposition in *C. elegans*

Lipid deposition can reflect the lipid metabolism status in the *C. elegans* [20]. Interestingly, in the SV group, while the glucose levels decreased, lipid deposition showed a dose-dependent increase. Combining the results of gene pathway analysis, the deposition of lipids may be associated with the downregulation of catabolic metabolism pathway. In the mice experiment, downregulation of catabolic metabolism resulted in increased insulin levels, stimulating lipid deposition in the organisms

[20]. Another nematode experiment showed that a decrease rate in carbohydrate metabolism, a type of catabolism, led to an increase in intracellular lipid deposition [21]. Therefore, it can be inferred that the downregulation of catabolic metabolism may contribute to the elevated lipid deposition in nematodes.

On the other hand, in the AP group, lipid deposition in *C. elegans* exhibited a dose-dependent decrease. Based on the results of gene pathway analysis, the reduction in lipids may be associated with the upregulation of *lipid metabolism pathways* and *oxidative phosphorylation pathway*. Previous research has shown that AP can enhance mitochondrial respiration in obese mice, thereby increasing energy synthesis and resulting in a reduction in intracellular lipids [6]. Another human study indicated a positive correlation between the upregulation of the *oxidative phosphorylation pathway* and weight loss [22], suggesting that AP may reduce lipid levels by promoting energy generation in *C. elegans*.

4.5. The safety of sweeteners on lipid and carbohydrate metabolism

Notably, AP groups showed an increase in relative glucose levels while decreasing lipids deposition. In contrast, SV exhibited an increase in lipids deposition while reducing relative glucose levels. In a human study comparing SV, AP, and sucrose, SV groups exhibited relatively better hypoglycemic effects compared to AP and sucrose [18]. Although direct comparative human experiments on the lipid aspect between SV and AP are lacking, numerous animal studies have suggested the potential of SV to promote lipid deposition in organisms [6], [7]. Thus, personalized recommendations regarding sweetener types may be beneficial for different population groups. For instance, AS may be suitable for diabetes patients, and SV may be suitable for obese patients.

In the study, all indicators in C. elegans exhibited a dose-dependent increase or decrease, demonstrating the importance of dosage in ensuring the safety of consumption, and highlight the need for future research to explore sweetener dosages and investigate the varying effects of different sweeteners on the human body.

4.6. The significance of this study

Prospective cohort study involving 15,486 men and women demonstrated a positive relationship between the consumption of sweetened beverages and the risk of developing type 2 diabetes and cardiovascular disease. Other studies have shown that the consumption of beverages containing natural sweeteners is associated with increased visceral adiposity and lipid levels, as well as a decrease in insulin sensitivity in obese individuals.

Additionally, in previous human studies, a genome-wide association study (involving 64,949 Europeans) revealed a positive association between the addition of artificial sweeteners to coffee and an increased risk of high-grade and low-grade serous ovarian cancer [23]. Similarly, the addition of artificial sweeteners to tea was positively associated with the risk of oral cavity and pharyngeal cancers.

C. elegans exhibits highly conserved metabolic pathways with humans. In terms of carbohydrate metabolism, *C. elegans* shares highly conserved pathways with humans, including glycolysis, the citric acid (TCA) cycle, galactose metabolism. Similarly, in lipid metabolism, *C. elegans* demonstrates conservation in pathways such as lipolysis (hormone-sensitive lipase), mitochondrial β-oxidation, fatty acid synthesis, and triacyl glyceride synthesis, which are also present in humans. The energy metabolism pathways, including oxidative phosphorylation and ATP synthesis, are highly conserved between *C. elegans* and humans as well. Additionally, there are similar insulin pathways in *C. elegans* compared to humans. Previous studies have utilized *C. elegans* as a reliable model organism to investigate metabolic dysfunctions and obesity, further highlighting its suitability for studying carbohydrate and lipid metabolism [9]. This study provides new insights into the dietary health and safety of artificial sweeteners and natural sweeteners.

5. Conclusion

Incorporating physiological and biochemical data, SV and AP have different impacts on *C. elegans* metabolism pathways. On the one hand, AP showed a dose-dependent increase in relative glucose levels while decreasing lipids deposition. On the other hands, SV exhibited a dose-dependent increase in lipids deposition while reducing relative glucose levels. Interestingly, SV and glucose exhibit many similarities in their effects on the metabolic pathways of *C. elegans*, which implies that SV poses risk in health issues. This experiment undoubtedly lays the foundation for future research on molecular mechanisms, such as how SV regulates carbohydrate and lipid metabolism in the body. This study provides preliminary exploration of the effects of AP and SV on nematode glycolipid metabolism, and points the way for future research on the molecular mechanisms involved. People who consume sugar substitutes should strive to minimize their usage and, secondly, choose sweeteners that are suitable for their health circumstances.

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