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Effect of Vitamin D and Calcium Supplementation on Insulin Resistance

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ABSTRACT

As people spend more time indoors, Vitamin D deficiencies have increased drastically due to less sunlight exposure. New studies have linked Vitamin D deficiencies to calcium deficiencies since Vitamin D helps the human body absorb calcium properly. Calcium regulates insulin sensitivity, and calcium deficiencies reduce insulin sensitivity, indicating how Vitamin D deficiencies can lead to insulin resistance. I tested the individual and combined effects of calcium and Vitamin D supplementation on insulin resistance in *Drosophila melanogaster*, using physical activity to measure improvement in insulin resistance. There were 3 identical trials, and 5 vials in each trial. The control group was fed with only drosophila medium. The remaining vials had 3 grams of table sugar mixed with the medium. The second vial had no additions. The third vial had an addition of liquid calcium, and the fourth vial had Vitamin D instead. The last group had both supplements. After a week, the peristalsis contractions of each group's larvae were counted in 30-second intervals. Additionally, adult flies from different groups were used for a climbing assay to measure the short-term impacts of these supplements on physical activity. Both measures yielded similar results: there was a visible increase in climbing performance and the number of peristalsis contractions in vials with supplementations compared to those without. The groups fed with individual supplements saw some increase, but the group with both supplements saw the most significant increase relative to sugar supplementation alone, supporting the hypothesis that calcium and Vitamin D supplementation improves insulin resistance.

Introduction

Over 42% of the population is deficient in vitamin D, a fat-soluble nutrient that is important in the retention of calcium and phosphorus, reducing cell cancer growth, controlling infections, and reducing inflammation (1). While this alone is alarming, it becomes more concerning as each of the effects are individually examined. Vitamin D plays a crucial role in calcium absorption, meaning that vitamin D deficiencies are synonymous with calcium deficiencies. Calcium stimulates and regulates insulin secretion, and so being calcium deficient decreases insulin sensitivity, making the hormone less effective (2). This condition, insulin resistance is characterized by muscle, fat, and liver cells' inability to take in glucose, resulting in high levels of blood glucose, and is a hallmark for Type II Diabetes (3). Additionally, vitamin D deficiencies also lower insulin sensitivity due to its modulating the effects of cytokines (4).

Drosophila melanogaster (*D. melanogaster*) has been extensively used as a model for insulin resistance and diabetes previously, and insulin resistance in *D. melanogaster* is made up of several *Drosophila* insulin-like peptides (DILPs), that activate a unique membrane receptor (6). High sugar diets (HSD) are a method used to induce insulin resistance in *D. melanogaster*. An HSD causes a severe growth inhibition as a consequence of peripheral DILP-resistance, giving rise to several metabolic disorders, including insulin resistance. This experiment utilized *D. melanogaster* to model insulin resistance caused by the HSD, and tested the effects of Vitamin D and calcium supplementation implemented through dietary means. The results indicated that the larvae in the containers with the addition of these supplements exhibited higher physical activity rates than the larvae with only a HSD, supporting the hypothesis that the dietary nutrient supplementation would improve insulin resistance.

Methods

To test my hypothesis and measure the effects of nutrient supplementation, five groups of D. melanogaster were set up. Each group had a different diet, made up of combinations of table sugar, drosophila medium, liquid vitamin D, liquid calcium. The table sugar represented the high sugar diet. The first group, was fed with only drosophila medium, acted as one of the control groups, kept for comparison to the experimental groups. The second group, also a control, was fed with drosophila medium, and the table sugar HSD. The third group was fed with drosophila medium, table sugar, and liquid vitamin D. The fourth group was fed with drosophila medium, table sugar, and liquid calcium. Finally, the last group was fed with drosophila medium, table sugar, liquid calcium, and liquid vitamin D. Approximately 0.6 mL of vitamin D and 0.667 mL of calcium was put into each group that required these drops. This number was found using the daily suggested human intake for both nutrients. After setting up the diets in each group, holes were poked in the lids of the plastic containers to allow the *D. melanogaster* to breathe. Then, the *D. melanogaster* in the original vials were anesthetized, and 15 drosophila were transferred into each group. The containers were then left in a dry place at about 28°C for 7 days, to allow *D. melanogaster* larvae to form and develop.

The modes of measurement used were peristalsis contractions and a climbing assay. After the seven-day development period, 10 *D. melanogaster* larva were collected from each group and the number of peristalsis (full-body) contractions in a 30-second period. They were placed in petri dishes with a yeast and water solution under a 40x magnification microscope. The adult *D. melanogaster* in each container was also utilized in a climbing assay, with 35 flies from each group. Placed in a vial marked with 3 cm and 6 cm markings, the number of *D. melanogaster* that crossed each mark from each group was noted.

Results

Across all three trials run of the experiment, similar results were observed. In each group, the highest number of peristalsis contractions was measured in the group without a HSD, fed with only drosophila medium. However, the least number of peristalsis contractions, on average, was measured in the group fed with only drosophila medium and table sugar. All the groups with additional vitamin D or calcium supplementation, or both, displayed a visible increase in the number of larval peristalsis contractions. The group fed with an addition of both supplements had the second most amount of peristalsis contractions, reflecting the greatest improvement despite the HSD. The climbing assay showed that the groups with calcium, vitamin D, or both supplements had the most number of drosophila crossed the 6-centimeter mark at the end of the 15-second period. Similar to the peristalsis contractions comparison, the most number of drosophila that crossed the 6 cm mark was from the group fed with only drosophila medium, but the groups with additions of calcium, vitamin D, or both had increasing number of flies cross the mark, in that respective order.

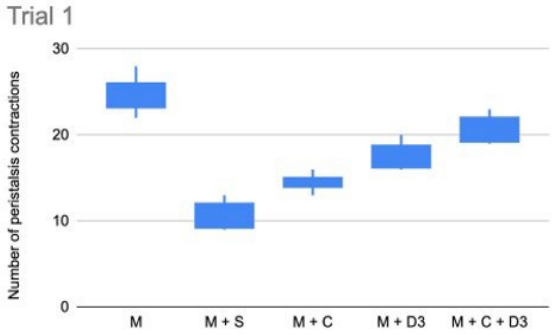

Figure 1. Using a box plot to describe the data collected by counting the number of peristalsis contractions for the larvae in Trial 1. There were 10 measurements for each of the categories, so a total of 50 measurements were taken for each trial.

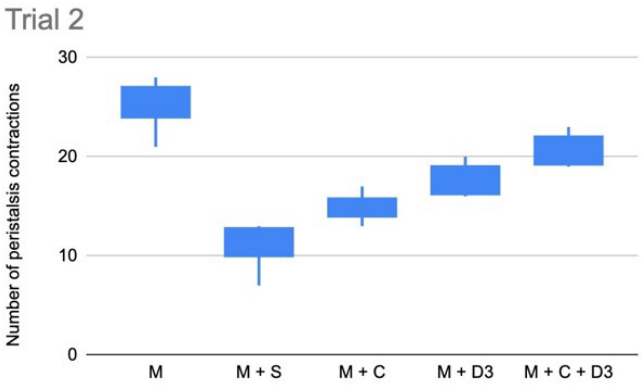

Figure 2. Using a box plot to describe the data collected by counting the number of peristalsis contractions for the larvae in Trial 2.

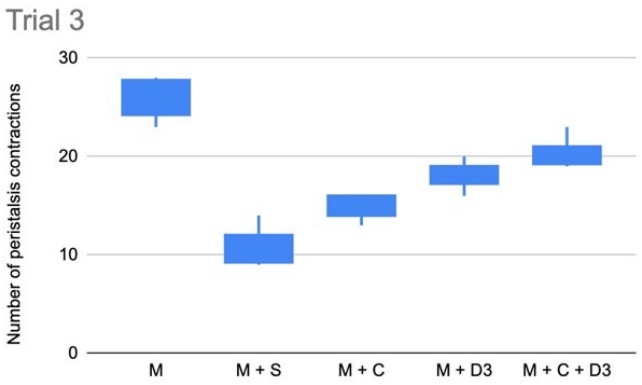

Figure 3. Using a box plot to describe the data collected by counting the number of peristalsis contractions for the larvae in Trial 3.

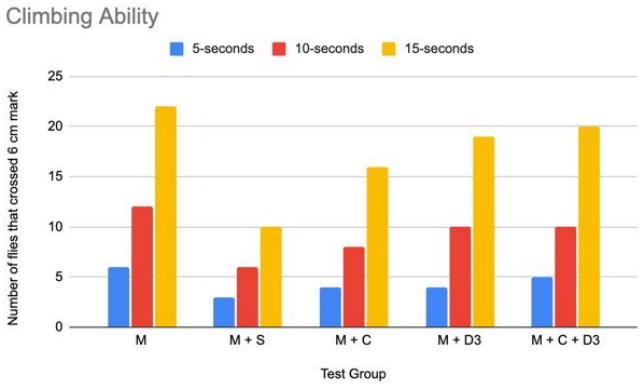

Figure 4. The number of drosophila that could climb past the 6 centimeters mark on a culture vial. Every 5 seconds, the number of drosophila that crossed the 6 centimeter mark were recorded. There were a total of 35 drosophila in each vial for each measurement.

Discussion

My hypothesis that the supplementation of calcium and vitamin D would improve insulin resistance in D. melanogaster fed with a HSD was supported by the results of the experiment. There were two modes of measurement used in this experiment: peristalsis contractions of the D. melanogaster larva and the climbing assay utilizing adult D. melanogaster. These measures used different stages in the D. melanogaster's life cycle to increase the reliability of the conclusions drawn. Both measures reflected that the treatment improved insulin resistance. When compared to the group fed with only drosophila medium, the other groups showed various percentage decreases in the number of peristalsis contractions (Table 1). However, though all the treatment groups had a lower number of contractions than the first group, they all had a significant improvement compared to the second control group fed with drosophila medium and artificial sweetener. The climbing assay also showed that more flies from the treatment groups crossed the 6 cm centimeter mark, supporting the hypothesis.

Table 1. The decrease in average number of larval peristalsis rates for each drosophila diet relative to the control.

	M + S	M + C	M + D3	M + C + D3
Trial 1	56.5%	41.9%	28.6%	16.1%
Trial 2	56.7%	41.2%	29.4%	17.5%
Trial 3	57.2%	42.8%	28.8%	20.6%

Conclusion

My hypothesis that the insulin resistance of Drosophila would improve because of calcium and Vitamin D supplementation was supported by the evidence collected. The statistical significance of the data was found using the Analysis of Variance (ANOVA) test, which suggested that the data is statistically significant for all three trials, $p < 0.00001$. The p-value indicates the chance that the correlation in the data is due to randomness rather than the treatment, so the lower the p-value, the more likely the treatment was effective in the experiment. The data shown in the results section indicates that the calcium and Vitamin D supplementation improved insulin resistance separately, but together, they had the greatest effect, which makes sense, given that Vitamin D boosts calcium absorbency, increasing insulin sensitivity more than either supplementation by itself. Since there were three trials and the results were similar amongst all the trials, the reliability of the results found increases, offering confidence in the data. The findings of this experiment are important because it indicates how expansive the effects of vitamin deficiencies are, leading to conditions and diseases that we may have thought were completely unrelated. Knowing the damaging effects of these deficiencies will allow us to target them and make sure we are taking the right supplements to avoid additional contributions to the development of the associated diseases and conditions.

Limitations

One possible limitation in the accuracy of my findings was the temperature at which the drosophila grew under. For optimal drosophila growth and reproduction, the drosophila should be at a place between 25°C and 28°C, but due to the experiment not taking place in a controlled school laboratory setting, the temperature range changed throughout the day, sometimes going above and below it. However, all the experimental groups of flies throughout all trials experienced the same temperature for the same amount of time.

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