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Original Article

Effect of *Citrus aurantiifolia (Key lime)* Extract on Postprandial Glycaemic Response of Oral Glucose Solution in Healthy Individuals

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Abstract

Background: Type 2 diabetes is becoming highly prevalent worldwide, prompting researchers to explore new treatments with potential hypoglycemic effects. *Citrus aurantiifolia* is a commonly found fruit in Sri Lanka, which exhibits various pharmacological properties, including antidiabetic potential.

Objective/s: This study aimed to evaluate the potency of *Citrus aurantiifolia* extract as a postprandial glycemic response-reducing agent in healthy individuals, exploring the potential benefit of key lime as a home remedy for type 2 diabetes.

Methods: In a randomized clinical trial (KIU/ERC/23/190), 74 healthy male participants were enrolled. Participants were randomly assigned into two groups and were administered 75g glucose in water, following an overnight fast of 8-10 hours. Subsequently, one group received *Citrus aurantiifolia* extract (Test group), while the other did not (Control group). Blood glucose levels were assessed in mmol/L at fasting, 30 minutes postprandially, and at regular intervals within one hour after ingestion of the glucose solution.

Results: The results revealed a significant attenuation of postprandial glycemic levels in individuals administered with lime extract compared to the control group. The mean blood glucose of the test group and the control group at 30-minute intervals were recorded as 3.17 mmol/L and 6.09 mmol/L respectively. The extract exhibited a significant reduction of 47.9% in blood glucose levels in the test group compared to the control group after 30 minutes, $p<0.05$.

Conclusion: Results suggest that *Citrus aurantiifolia* extract possesses potential antihyperglycemic properties. Integrating lime extract as a dietary supplement presents a cost-effective approach to home-based glycemic control and may be beneficial in management of type 2 diabetes.

Keywords: *Citrus aurantiifolia*, Diabetes mellitus, Postprandial glycemic response, Type 2 diabetes prevention

Introduction

Type 2 diabetes mellitus (T2DM) represents a critical global health emergency, as evidenced by the alarming rise in its prevalence and associated morbidity, mortality, and economic burden. According to the International Diabetes Federation (IDF), approximately 463 million adults aged 20-79 years were living with diabetes worldwide in 2019, with projections indicating a rise to 700 million by 2045 (Fazakerley et al., 2019). T2DM accounts for the majority of diabetes cases, with around 90% of individuals affected having this form of the disease. The consequences of uncontrolled T2DM are severe and far-reaching. In Sri Lankan context, the pooled T2DM prevalence was the highest in the latest period of 2011-2021 (17.25%) than in the period of 2000s (11.84%) and 1990s (5.62%) (Akhtar et al., 2023). They further stated that the growing trend of diabetes and pre-diabetes over the last 30 years is alarming in Sri Lanka.

Postprandial blood sugar is the measurement of blood glucose levels after eating, revealing how the body processes carbohydrates and uses insulin. The term "postprandial glycemic response" describes the rise in blood sugar that occurs after eating, and it has been suggested that frequent postprandial glucose excursions pose a significant risk for the development of insulin resistance (Ogiso et al., 2022; Schofield & Sutherland, 2012). Insulin resistance is a metabolic condition where the body's cells fail to respond effectively to insulin, leading to elevated blood sugar levels. This condition, where normal insulin levels are insufficient to elicit a normal response, results in hyperglycaemia. It is the primary cause of type 2 diabetes mellitus (T2DM), often called "insulin-independent" diabetes, which is more prevalent (Blaak et al., 2012; Pattaranit et al., 2008). To compensate for the resistance, the pancreas produces more insulin. Initially, this can maintain normal blood sugar levels, but over time, the pancreas may struggle to keep up with the demand for insulin. The insulin resistance worsens chronically elevating blood sugar. This results in prediabetes or eventually progressing to full-blown type 2 diabetes (Mokhehle, 2014).

In recent years, there has been growing interest in exploring natural remedies and dietary interventions to combat the rising incidence of diabetes and associated complications (Choudhury et al., 2017). Citrus species, which include fruits like oranges, grapefruits, lemons, and limes, have been studied for their potential effects on glycemic response, particularly concerning their impact on blood sugar levels. Citrus fruits are a good source of dietary fibre, particularly soluble fibre such as pectin. Fiber can help slow down the absorption of sugar from the digestive tract, leading to a more gradual rise in blood glucose levels after consumption. Citrus fruits are rich in flavonoids and antioxidants, which may have potential benefits for glycemic control (Visvanathan & Williamson, 2021). Some studies suggest that these compounds could improve insulin sensitivity and reduce inflammation, both of which are factors that can influence blood sugar regulation. One such natural product under investigation is *Citrus aurantiifolia*, commonly known as Key lime. Key lime, which is a small, round citrus fruit is native to Southeast Asia. It belongs to the Rutaceae family and is characterized by its unique flavor profile, combining a tangy and slightly sweet taste. It is a rich source of essential nutrients, including vitamin C, vitamin A, dietary fibre, and various phytochemicals such as flavonoids and limonoids (Narang & Jiraungkoorskul, 2016).

In Sri Lanka, there are almost no previous studies done to evaluate key lime’s ability to lower postprandial glucose absorption which creates a vast literature gap. *Citrus aurantiifolia* is a fruit that can be easily obtained in Sri Lanka and has a lot of health benefits, but it is yet to be used as medicine against type 2 diabetes. Therefore, this study aims to explore the existing scientific evidence surrounding the potential effects of key lime on postprandial glycemic control. Through a comprehensive analysis of available research, we attempted to elucidate the mechanisms underlying key lime's influence on these interconnected pathways and evaluate its therapeutic potential as a natural intervention for glycemic management, insulin resistance and ultimately T2DM.

Methods

Study design and study population

A randomized clinical trial was conducted investigating 74 male participants with mostly similar baseline characteristics, within the age of 21 – 30, and BMI in between the healthy range of 18.5 to 24.9 kg/m^2. This population was selected according to the criteria that, with the aging process (Broughton & Taylor, 1991) and when the BMI is not in healthy range (Taylor, 2012), the insulin resistance will be altered in the individual, affecting the current data. Also, women participants were excluded from the study due to the possible alterations in hormones affecting the insulin resistance throughout the month due to menstrual cycle, which will affect the blood glucose regulation after glucose consumption (Geer & Shen, 2009).

Study Setting

Data were collected from July 2023 to April 2024 at different data-collection centres at Colombo, Anuradhapura and Kandy in Sri Lanka.

Data collection

Preparation of glucose drink

The glucose drinks for the participants were made from commercially available glucose powder, (Morison Glucomile 100% glucose monohydrate) and water. A glucose solution was prepared with 75g of glucose powder by fully dissolving in 250ml of water (Saeedi et al., 2019).

Preparation of lime extract

The lime extracts were made at the site by extracting 100ml of lime extract with an extracting tool. The extract was given fresh to the selected participants to avoid any changes due to environmental factors with time exposed to the air. The raw lime extract was used for this purpose, which means there was no any other ingredient added to the extract produced from the lime fruits.

Participant Recruitment and Screening

Participants were recruited randomly after providing informed consent. Initially, participants read the information sheet and completed the details about age, health status, and willingness to participate. Only those without a history of severe gastric attacks and who were not on regular medications were selected.

BMI Measurement and Selection

Eligible participants underwent BMI assessment. Height and weight were measured using a measuring tape and weight scale, respectively. BMI was calculated using a web application, and only participants with a BMI within the normal range (18.5–24.9 24.9 kg/m^2) were included for further testing (*BMI Calculator*, n.d.).

Fasting Blood Glucose Testing

Participants were instructed to fast for 12 hours before testing. Fasting blood glucose levels were measured using the finger-prick method with the Accu-Chek glucometer. Participants with fasting blood glucose levels between 70 mg/dL and 100 mg/dL were selected for the study.

Group Assignment

Participants were assigned to either the control group (n = 37) or the test group (n = 37) based on their order of participation. The first five participants were assigned to the control group, followed by the next five participants to the test group, with this alternating pattern continuing until all participants were allocated.

Intervention

Each participant consumed an oral glucose solution first. Participants in the control group were then given 100ml of water, while participants in the test group received 100ml of raw lime extract.

Blood Glucose Monitoring

Blood glucose levels were measured at three time points post-consumption using the Accu-Chek glucometer as follows: 30 minutes after consumption, 45 minutes after consumption, 60 minutes after consumption. These steps ensured consistency and accuracy in assessing the effects of the interventions on blood glucose levels.

Data Analysis

The blood glucose levels from groups 1 and 2 were compared by calculating the mean values for each group at each blood glucose checkpoint, ranging from fasting blood glucose to the fourth postprandial measurement. The data were systematically organized in an Excel database and imported into SPSS 23 (Statistical Package for Social Sciences) for statistical analysis. A T-test was performed to calculate the p-values for each result set, determining the significance of differences between the groups.

Results

1. Anthropometric data of the participants

Anthropometric data encompasses measurements of the human body, providing crucial insights into various aspects of health, nutrition, and physical fitness. Accordingly, the mean weight, height and BMI of the participants were 65.91kg ± 8.04, 1.70m ± 0.07m and 22.52 ± 2.04, respectively. Additionally, the mean age was 25 ± 2 in years.

2. Blood Glucose results mean values

The mean values of the control group and the test group were graphed as shown in Figure 1. The values of the test group ran below the values of the control group, indicating a lower level of blood glucose. With mean values of 57.2 mg/dl in the test group and 109.86 mg/dl in the control group at 30-minute intervals. The extract exhibited a significant reduction of 47.9% in blood glucose levels in the test group compared to the control group after 30 minutes.

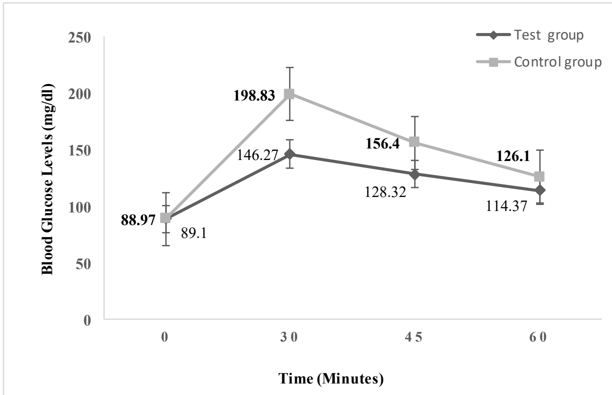

Figure 1: Comparison of mean values of blood glucose obtained for the control group and test group at different time intervals

Comparing mean blood glucose values of the control group and the test group after 30 minutes time interval using t- test in SPSS application resulted in $p = 0.000$, $p < 0.05$. Independent t-test resulted in $p = 0.000$ ($p < 0.05$) for the 45-minutes data. And for the 60 minutes data $p = 0.003$, ($p < 0.05$). This indicates that the blood glucose elevation is higher in the control group than in the test group not only after 30 minutes but also after 45 minutes and 60 minutes.

3. Correlation between BMI values and fasting blood glucose values

The correlation between BMI values and fasting blood glucose levels is well-established, with higher BMI often associated with elevated fasting blood glucose levels. A correlation of 0.928 was reported with $p = 0.000$, $p < 0.01$, indicating a strong positive relationship between rising BMI and fasting blood glucose.

Figure 2: Correlation between BMI values and fasting blood glucose values

Discussion

The study aimed to determine the effect of *Citrus aurantiifolia* on postprandial glycemic response in healthy males and to determine glycemic response of oral glucose solution in different time intervals in healthy males. In this study, *Citrus aurantiifolia* demonstrated a significant reduction on the postprandial glycaemic response compared with the control blood glucose values, mainly after 30-minute of ingestion, resulting in a p-value of $p < 0.05$, indicating the potential to be used as a substance to reduce the risk of developing type 2 diabetes. The absorption of blood glucose at 45-minute interval and after 60 minutes, also showed significances of $p < 0.05$. The 30-minute interval was considered as the main point to compare the effect of *Citrus aurantiifolia* on postprandial blood glucose and analysis for the study since it was the peak of post prandial blood glucose curves in both groups, the control and the test group.

This pattern shown in the current study where the post prandial blood glucose level reached the highest peak after 30 minutes of ingestion, is consistent with the results in most of the general oral glucose tolerance tests and other studies such as the research done by Crummett and Grosso in 2022. In that study, mean incremental blood glucose values were subtracted from baseline blood glucose values of 20 participants over 60 minutes, after consuming either whole fruit or blended fruit, which again has shown that the postprandial blood glucose does peak after about 30 minutes (Crummett & Grosso, 2022). In the current study, after 30 minutes, post prandial blood glucose peaked with an average value of 198.83 ± 43.70 mg/dl in the control group. In contrast to that, post prandial blood glucose level of the test group elevated only up to mean value of 146.27 mg/dl after 30 minutes, showing a significant reduction in the blood glucose values.

In a different aspect, blood glucose elevation after 30 minutes was also calculated separately for the test group and the control group by subtracting the baseline fasting blood glucose level of each individual from their postprandial blood glucose after 30 minutes, 45 minutes, and 60 minutes. At 30-minute interval, the calculated results revealed a significant attenuation of postprandial glycemic response in individuals administered with *Citrus aurantiifolia* extract compared to the control group with mean values of 57.2 mg/dl in the test group and 109.86 mg/dl in the control group. The extract exhibited a significant reduction of 47.9% in blood glucose levels in the test group compared to the control group after 30 minutes.

In a study conducted at the College of Health Sciences, Nigeria obtained results that suggest the presence of polyphenols such as flavonoids in lime can be responsible for the reduction in plasma glucose concentration (E et al., 2021). In their study, they got significant results including a drastic drop in blood glucose concentration in albino rats, when *Citrus aurantiifolia* was administered. As Ramya et al. (2020) explained, *Citrus aurantifolia* also gives anti-diabetic actions (Ramya et al., 2020). And Mawarti et al. (2018) showed its ability to lower glycemic levels demonstrating a significant impact of the *C. aurantifolia* and *C. burmannii* extracts on blood glucose levels ($p = 0.035$) (Mawarti et al., 2018). These several outcomes align with the current study indicating the possibility of administrating key lime extract as a diabetic controlling agent.

Gandhi et al. (2020) provided more information describing that, among the flavonoids found in *Citrus aurantiifolia*, quercetin is the most important substance. It possesses a wide range of medicinal qualities, such as anti-inflammatory, antinociceptive, and anticancer effects. For this reason, quercetin is regarded as the most significant citrus flavonoid due to its capacity to regulate the key inflammatory mediators associated with metabolic disorders such as type 2 diabetes (Gandhi et al., 2020). Dhanya et al. (2017), explaining the mechanism of how quercetin from lime can facilitate the insulin signalling pathway and increase the glucose uptake from cells, indirectly lowering the frequencies of blood glucose spikes, which clarifies the findings of the current research (Dhanya et al., 2017). Therefore, this could be one of the components of key lime responsible for the results obtained from the current study where the blood glucose levels were reduced compared to the control values in a particular time period.

For this study, female participants were not enrolled since their hormonal changes which take place throughout the month affect insulin resistance and insulin sensitivity, which can affect the results of the current study by altering how insulin acts on blood glucose and change the levels of blood glucose (Geer & Shen, 2009). There is a complex relationship between oestrogen and insulin resistance, and in premenopausal women, oestrogen may enhance the body's ability to use insulin effectively, helping to maintain stable blood sugar levels. As De Paoli et al. (2021) suggest, metabolic disturbances, such as insulin resistance, tend to dramatically increase with the onset of menopause, and oestrogen replacement therapy significantly reduces the risk of metabolic syndrome (De Paoli et al., 2021). Therefore, in males blood glucose changes can be more fairly identified while the females are excluded from the study since their menstrual phases cannot be determined to have an idea about the oestrogen levels which has an effect on insulin sensitivity. Elderly subjects were not enrolled since insulin resistance can develop with age. A reduction in the expression of skeletal muscle glucose transporters (GLUTs), glucose transporter type 1 (GLUT1) and glucose transporter type 4 (GLUT4) in older age can reduce the glucose uptake to skeletal muscle (Santos et al., 2011).

Also, obese persons were not enrolled since insulin resistance is high in obese subjects (Wondmkun, 2020; Ye, 2013). A study using fibre-enriched orange juice (*Citrus sinensis)* which has similar characteristics as key lime, has shown that the consumption of orange juice modifies the time course of glucose and insulin (Bosch-Sierra et al., 2019). In a research study conducted by De Paiva et al. (2019) using adult men and women (n = 36), both lean and obese subjects were investigated. The study focused on the effects of commercially processed orange juice (processed OJ) and freshly squeezed orange juice (fresh OJ) on insulin and blood serum glucose. The results demonstrated that consuming any kind of orange juice enhanced the antioxidant capacity of study participants in comparison to consuming sugary beverages. It also showed that orange juice delayed glucose absorption and decreased insulin production, indicating effective glucose clearance. In their research, for lean subjects compared to obese subjects, these effects of Citrus spp., were on blood glucose related parameters were significantly lowered (De Paiva et al., 2018).

The positive correlation between BMI values and fasting blood glucose levels is rooted in the complex interplay of adiposity, insulin resistance, and glucose metabolism. A positive correlation between BMI and fasting blood glucose was found in the study done using a total of 42 elderly persons from an area in southern Estonia (Sepp et al., 2014). A limitation of this study could be the small sample size, yet the data collected from each participant has shown an identical pattern, therefore can lead to an assumption that the data is sufficient to generate the current conclusion.

Conclusion

In conclusion, key lime extract significantly reduced blood glucose levels following the ingestion of an oral glucose solution, providing compelling evidence for the beneficial effects of *Citrus aurantiifolia* extract on postprandial glycemic response in healthy individuals and for an exact dosage regimen, further studies are encouraged. This attenuation of postprandial hyperglycaemia suggests that *Citrus aurantiifolia* extract may serve as a valuable dietary adjunct for individuals aiming to mitigate fluctuations in blood glucose levels and promote metabolic health.

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Conflict of Interest

There are no conflicts of interest.

References

Akhtar, S., Ali, A., Asghar, M., Hussain, I., & Sarwar, A. (2023). Prevalence of type 2 diabetes and pre-diabetes in Sri Lanka: a systematic review and meta-analysis. *BMJ Open*, *13*(8), e068445. https://doi.org/10.1136/bmjopen 2022-068445

Blaak, E. E., Antoine, J. M., Benton, D., Björck, I., Bozzetto, L., Brouns, F., Diamant, M., Dye, L., Hulshof, T., Holst, J. J., Lamport, D. J., Laville, M., Lawton, C., Meheust, A., Nilson, A. E., Normand, S., Rivellese, A. A., Theis, S., Torekov, S. S., & Vinoy, S. (2012). Impact of postprandial glycaemia on health and prevention of disease. *Obesity Reviews*, *13*(10), 923–984. https://doi.org/10.1111/j.1467-789x.2012.01011.x

BMI calculator. (n.d.). https://www.calculator.net/bmi-calculator.html

Bosch-Sierra, N., Marqués-Cardete, R., Gurrea-Martínez, A., Valle, C. G., Talens, C., Alvarez-Sabatel, S., Bald, C., Morillas, C., Hernández-Mijares, A., & Bañuls, C. (2019). Effect of Fibre-Enriched orange juice on postprandial glycaemic response and satiety in healthy individuals: an acute, randomised, Placebo-Controlled, Double-Blind, crossover study. *Nutrients*, *11*(12), 3014. https://doi.org/10.3390/nu11123014

Broughton, D., & Taylor, R. (1991a). Deterioration of Glucose Tolerance 117 with Age: The Role of Insulin Resistance. *Age And Ageing, 20*(3), 221 225. https://doi.org/10.1093/ageing/20.3.221

Choudhury, H., Pandey, M., Hua, C. K., Mun, C. S., Jing, J. K., Kong, L., Ern, L. Y., Ashraf, N. A., Kit, S. W., Yee, T. S., Pichika, M. R., Gorain, B., & Kesharwani, P. (2017). An update on natural compounds in the remedy of diabetes mellitus: A systematic review. *Journal of Traditional and Complementary Medicine*, *8*(3), 361–376. https://doi.org/10.1016/j.jtcme.2017.08.012

Crummett, L. T., & Grosso, R. J. (2022). Postprandial Glycemic Response to Whole Fruit versus Blended Fruit in Healthy, Young Adults. *Nutrients*, *14*(21), 4565. https://doi.org/10.3390/nu14214565

De Paiva, A., Gonçalves, D. R., Ferreira, P. S., Baldwin, E. A., & Cesar, T. B. (2019). Postprandial effect of fresh and processed orange juice on the glucose metabolism, antioxidant activity and prospective food intake. *Journal of Functional Foods*, *52*, 302–309. https://doi.org/10.1016/j.jff.2018.11.013

De Paoli, M., Zakharia, A., & Werstuck, G. H. (2021). The role of estrogen in insulin resistance. *American Journal of Pathology*, *191*(9), 1490–1498. https://doi.org/10.1016/j.ajpath.2021.05.011

Dhanya, R., Arya, A. D., Nisha, P., & Jayamurthy, P. (2017). Quercetin, a Lead Compound against Type 2 Diabetes Ameliorates Glucose Uptake via AMPK Pathway in Skeletal Muscle Cell Line. *Frontiers in Pharmacology, 8*. https://doi.org/10.3389/fphar.2017.00336

E, K. B., James, E., & Prophet, P. T. (2021). Hypoglycaemic effects of decoction of Camelia sinensis (Lipton tea) and Citrus aurantifolia (Lime) on plasma glucose concentration and weight of normal albino rats. *Scholars International Journal of Biochemistry*, *4*(3), 20–25. https://doi.org/10.36348/sijb.2021.v04i03.001

Fazakerley, D. J., Krycer, J. R., Kearney, A. L., Hocking, S., & James, D. E. (2019). Muscle and adipose tissue insulin resistance: malady without mechanism? *Journal of Lipid Research*, *60*(10), 1720–1732. https://doi.org/10.1194/jlr.r087510

Gandhi, G., Vasconcelos, A. B., Wu, D., Li, H., Antony, P., Li, H., Geng, F., Gurgel, R., Narain, N., & Gan, R. (2020). Citrus flavonoids as promising phytochemicals Targeting diabetes and related complications: A Systematic review of in vitro and in vivo studies. https://www.semanticscholar.org/paper/Citrus-Flavonoids-as-Promising-Phytochemicals-and-A-Gandhi-Vasconcelos/00d56111b2d8f73746ef473af28858ddb29bb9d8

Geer, E. B., & Shen, W. (2009). Gender differences in insulin resistance, body composition, and energy balance. *Gender Medicine*, *6*, 60–75. https://doi.org/10.1016/j.genm.2009.02.002

Mawarti, H., Khotimah, M. Z. A., & Rajin, M. (2018). Ameliorative effect of <i&gt Citrus aurantifolia</i> and <i>Cinnamomum burmannii</i> extracts on diabetic complications in a hyperglycemic rat model. *Tropical Journal of Pharmaceutical Research*, *17*(5), 823. https://doi.org/10.4314/tjpr.v17i5.11

Mokhehle, M. (2014). Nutritional status, glycemic control and barriers to treatment compliance among patients with type 2 diabetes attending diabetes clinics in Maseru, Lesotho. https://scholar.ufs.ac.za/handle/11660/1896

Narang, N., & Jiraungkoorskul, W. (2016). Anticancer activity of key lime, *Citrus aurantifolia. Pharmacognosy Reviews*, *10*(20), 118. https://doi.org/10.4103/0973-7847.194043

Ogiso, K., Shayo, S. C., Kawade, S., Hashiguchi, H., Deguchi, T., & Nishio, Y. (2022). Repeated glucose spikes and insulin resistance synergistically deteriorate endothelial function and bardoxolone methyl ameliorates endothelial dysfunction. *PLOS ONE*, *17*(1), e0263080. https://doi.org/10.1371/journal.pone.0263080

Pattaranit, R., Van Den Berg, H., & Spanswick, D. (2008). The Development of Insulin Resistance in Type 2 Diabetes: Insights from Knockout Studies. *Science Progress*, *91*(3), 285–316. https://doi.org/10.3184/003685008x361343

Ramya, S., Narayanan, V., Ponnerulan, B., Saminathan, E., & Veeranan, U. (2020). Potential of peel extracts of Punica granatum and Citrus aurantifolia on alloxan-induced diabetic rats. *Beni-Suef University Journal of Basic and Applied Sciences*, *9*(1). https://doi.org/10.1186/s43088-020-00049-9

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., & Williams, R. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *157*, 107843. https://doi.org/10.1016/j.diabres.2019.107843

Santos, J. M. D., Benite-Ribeiro, S. A., Queiróz, G., & Duarte, J. A. (2011). The effect of age on glucose uptake and GLUT1 and GLUT4 expression in rat skeletal muscle. *Cell Biochemistry and Function*, *30*(3), 191–197. https://doi.org/10.1002/cbf.1834

Schofield, C. J., & Sutherland, C. (2012). Disordered insulin secretion in the development of insulin resistance and Type 2 diabetes. *Diabetic Medicine*, *29*(8), 972–979. https://doi.org/10.1111/j.1464-5491.2012.03655.x

Sepp, E., Kolk, H., Lôivukene, K., & Mikelsaar, M. (2014). Higher blood glucose level associated with body mass index and gut microbiota in elderly people. *Microbial Ecology in Health and Disease*, *25*. https://doi.org/10.3402/mehd.v25.22857

Taylor, R. (2012). Insulin Resistance and Type 2 Diabetes. Diabetes, *61*(4), 778–779. https://doi.org/10.2337/db12-0073

Visvanathan, R., & Williamson, G. (2021). Effect of citrus fruit and juice consumption on risk of developing type 2 diabetes: Evidence on polyphenols from epidemiological and intervention studies. *Trends in Food Science and Technology.* https://doi.org/10.1016/j.tifs.2021.06.038

Wondmkun, Y. T. (2020). <p>Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *13,* 3611–3616. https://doi.org/10.2147/dmso.s275898

Ye, J. (2013). Mechanisms of insulin resistance in obesity. *Frontiers of Medicine, 7*(1), 14–24. https://doi.org/10.1007/s11684-013-0262-6