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Effect of less refined sugar (LRS) *vs*. refined sugar (RS) on postprandial glycaemic responses, glycaemic profile, and lipid profile, in normal and type 1 diabetic rats: A preliminary study

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Abstract

Less refined sugar (LRS) is a less refined natural sweetener that retains some polyphenols and minerals naturally occurring in the sugarcane, and its metabolic impact still needs to be studied. The present work compared the glycaemic response of three doses (Low, L; Medium, M; and High, H) of LRS and refined sugar (RS), in 54 normal and STZ-induced diabetic rats (n = 6/group). The metabolic response was also evaluated after two weeks of acute intervention in diabetic rats. The peak of glycaemia and global glucose response was lower after oral LRS administration than RS at corresponding doses, in normal and type 1 diabetic rats. After two weeks of interventions, fasting serum glucose level was found to be lower but not significant ($p > 0.05$) for groups receiving LRS-L, LRS-M, and LRS-H (at 11.53 ± 2.51, 12.68 ± 5.09, and 14.88 ± 1.46 mmol/L, respectively) in comparison with a corresponding dose of RS-L, RS-M, and RS-H (at 12.48 ± 0.74, 15.02 ± 1.28, and 15.70 ± 0.05 mmol/L, respectively). Consumption of LRS showed lower insulin resistance (IR), as revealed by reduced fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR) compared to RS treatment groups. This suggested that the consumption of LRS could be a less harmful alternative to RS in normal and diabetic condition, which may encourage both the industry and the public to substitute RS with LRS in food preparation and products. However, further detailed research is recommended to conclude the outcomes.

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Introduction

Sugar typically refers to a category of simple carbohydrates that includes monosaccharides (fructose, glucose, and galactose) and disaccharides (sucrose, maltose, and lactose), which affect the body differently (Brouns, 2020). The most used form of sugar is sucrose (table sugar), a disaccharide of one part glucose and one part fructose (Brouns, 2020). Sucrose is natural and non-toxic, sweet-tasting, water-soluble crystalline, and derived mainly from beet or cane sugar (Singh *et al*., 2020). Excessive added sugar consumption has been implicated in obesity, metabolic disorders, diabetes, cardiovascular disease, cancers, depression, and cognitive impairment (Gillespie *et al*., 2023). Added sugars

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refer to all sugars added in food preparation or manufacturing. This definition is generally not considered to include sugars found naturally in honey, fruit juices, and syrups, nor sugars found within the cellular structure of foods, such as dairy foods, or the carbohydrates found in nuts, fruits, cereal grains, or vegetables (Gillespie *et al*., 2023).

Due to the mentioned health risks, several scientific organisations, including WHO, the UK National Health Service (NHS), and the American Heart Association (AHA), have recommended significant restrictions on the upper limits of sugar consumption (Buyken *et al*., 2018; Gillespie *et al*., 2023). The AHA and WHO recommend that not more than 10% of total calories be added sugars, approximately 200 calories (50 g) or 12 teaspoons for an average adult (Buyken *et al*., 2018; Gillespie *et al*., 2023). However, both these organisations recommended that a limit of 5% of total calories daily would improve health outcomes (Buyken *et al*., 2018; Gillespie *et al*., 2023).

Based on food availability data, added sugar intake attained the highest point of over 69 kg per person yearly in the USA in 1999 (Gillespie *et al*., 2023). Trends from the US 2001 to 2018 National Health and Nutrition Examination Survey (NHANES) highlighted a modest decline, although observed only in younger adults (aged 19 - 50 years), from a mean of 96.6 g to 72.3 g/day (DiFrancesco *et al*., 2022). Despite the reduction, global added sugar consumption is still high and well above the recommended 5 or 10% of daily energy intake. Indeed, reducing added sugar consumption is daunting as it is easy to underestimate its consumed amount daily. It was reported that two-thirds of packaged foods contain added sugars to enhance flavour or to extend shelf life. Sweets and soft drinks typically contain high levels of added sugar. However, even the "so-called" healthier food products, such as granola bars, cereals, and yoghurt, can also contain high amounts of added sugars (Valle *et al*., 2020).

The population's awareness of the recommendation of health agencies motivates consumers to find alternative healthier sweeteners like natural sweeteners, brown sugar (BS), unrefined and less refined sugar (LRS), maple syrup, honey, and others (Valle *et al*., 2020). Unrefined sugar and LRS alternatives may benefit health compared to refined sugar (RS), as shown by many researchers (Sánchez-Tapia *et al*., 2020; Azlan *et al*., 2020; 2023; Ebadi and Azlan, 2020; 2023; Shamsi-Goushki *et al*., 2021; Zidan and Azlan, 2022). Although phytochemical compounds and minerals are concentrated across sugarcane-product production, they are eliminated in RS production, and concentrated in the by-product, blackstrap molasses (Zidan and Azlan, 2022). Unrefined sugar and LRS retain minor components like minerals and phytochemical compounds, and has a superior nutritional value to RS due to the less refining processes. RS consists of nearly 98.33 - 99.63 g per 100 g of sucrose, while unrefined sugar consists of 88.46 - 89.85 g per 100 g of sucrose (Ebadi and Azlan, 2020). In this context, LRS is one of the most essential natural brown sweeteners (Azlan *et al*., 2023).

LRS is generally granulated, light brown sugar rich in vitamins, amino acids, minerals, and phytochemical compounds, but lower in sucrose due to the less refining processes (Azlan *et al*., 2023). Recently, it was found that LRS contains the highest phenolic compounds at 57.72 μg/g, compared to other sugars like BS and RS, which were at 42.19 and 22.06 μg/g, respectively (Azlan *et al*., 2023). Major polyphenolic compounds detected in LRS include *p*-coumaric, caffeic, syringic, and ferulic acids (Azlan *et al*., 2023). LRS showed inhibitory activity against α-amylase and α-glucosidase *in vitro*, but not RS, which was attributed mainly to LRS's content of caffeic and ferulic acids (Azlan *et al*., 2023).

A similar product of LRS, minimally refined brown sugar (MRBS), was categorised as a low glycaemic index (GI) sugar, while RS was categorised as a medium glycaemic index sugar (Azlan *et al*., 2022). High GI foods result in larger postprandial blood glucose excursions compared with low GI foods, and this is assumed to be responsible for adverse health outcomes through extended hyperinsulinemia and hyperglycaemia, causing long-term multi-organ stress (Campbell *et al*., 2018). Consumption of MRBS resulted in a better satiety profile, lower postprandial glycaemic profiles, and higher total antioxidant capacity values than RS in human subjects (Azlan *et al*., 2022). The contents of minerals, particularly potassium, manganese, and selenium, were higher in MRBS or other unrefined sugar than in RS (Azlan *et al*., 2020; Zidan and Azlan, 2022). Selenium acts as an antioxidant and insulin-mimetic nutrient (Fontenelle *et al*., 2018), which makes unrefined sugar and LRS ideal healthier choices for healthy and type 2 diabetic patients (Azlan *et al*., 2023).

All in all, substituting major refined sweeteners with unrefined sugar or LRS might be a practical non-pharmacological approach for preventing the development and progression of metabolic-related disorders that are connected to oxidative stress and inflammation (Zidan and Azlan, 2022; Ebadi and Azlan, 2023). Despite the well-documented nutritional profile and its promising effect on diabetes management, the efficacy of LRS consumption in preventing or improving metabolic-related disease has not been investigated yet (Azlan *et al*., 2020; 2023; Zidan and Azlan, 2022). Therefore, the present work assessed the glycaemic response of LRS and RS at different doses, in healthy and type 1 diabetic rats. The metabolic responses (glucose, insulin, and lipid profile) were also evaluated after two weeks of intervention in diabetic rats. The present work hypothesised that the intake of LRS may be beneficial for glucose regulation compared to RS.

Materials and methods

Sweetener

RS and LRS were purchased in triplicate from grocery stores of a particular brand in Malaysia.

Animal protocol

Sprague-Dawley male rats (n = 54) weighing 200 - 250 g were housed in two animals per cage, in a well-ventilated room, under controlled conditions (12:12 light: dark cycle, controlled temperature (23 ± 5 °C) and humidity), with *ad libitum* food and deionised water. All animals were fed with standard rat chow (Maintenance diet, 1320 M, Altromin Spezialfutter GmbH and Co. KG, Im Seelenkamp, Lage, Germany) containing 61% kcal from carbohydrates, 24% kcal from protein, and 15% kcal from fat. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (approval no.: UPM/IACUC/AUP-R031/2021). Maintenance and care of the experimental animals conformed to the International Guiding Principles for Animal Research. Calculation of sample size used Eq. 1 for the comparison of means (Charan and Kantharia, 2013; Sánchez-Tapia *et al*., 2020; 2019):

$$n = \frac{2s2(Z\alpha+Z\beta)2}{\Delta 2} \quad \text{(Eq. 1)}$$

where, n = sample size; s = standard deviation; Zα = Type I error (confidence level α = 0.05 corresponding to a value of Z = 1.96); Zβ = with a power of 80% (value of Z = 0.84); and Δ = difference in magnitude between means of the treatments (amplitude).

Experimental design Phase 1: Oral glucose tolerance test (OGTT) using RS and LRS on healthy rats

After 1 w of acclimatisation, an oral glucose tolerance test was conducted on 42 healthy rats. The rats were distributed into seven groups, with six rats per group. After overnight fasting, rats were gavage with different types of sugar solution based on the assigned groups as follows:

i. Group 1 (glucose, GL): received 2.6 g/kg of glucose solution (reference solution).
ii. Group 2 (less refined sugar low dose, LRS-L): received 2.6 g/kg of LRS.
iii. Group 3 (less refined sugar medium dose, LRS-M): received 5.2 g/kg of LRS.
iv. Group 4 (less refined sugar high dose, LRS-H): received 6.9 g/kg of LRS.
v. Group 5 (refined sugar low dose, RS-L): received 2.6 g/kg of RS.
vi. Group 6 (refined sugar medium dose, RS-M): received 5.2 g/kg of RS.
vii. Group 7 (refined sugar high dose, RS-H): received 6.9 g/kg of RS.

Glucose levels were measured before the administration of different solutions (0 h) and 15, 30, 60, 90, and 120 min after the sugar administration. A calibrated hand-held glucometer (Accu-chek Performa, Roche, Germany) was used to assess glycaemia at each point in time (St-Pierre *et al*., 2014). The low and medium doses were chosen based on the recommendation of WHO to restrict sugar consumption to 5 and/or 10% of total calories (WHO, 2015), equivalent to 25 and 50 g/day of human consumption, respectively. The high dose was chosen based on the latest statistics on daily human consumption (from sugary drinks, beverages, and sugar in food), equal to 70 g/day following the American Guideline 2020 - 2025 (Ricciuto *et al*., 2021). The animal equivalent doses were calculated based on body surface area according to Nair and Jacob (2016) using Eq. 2:

Animal Equivalent Dose (mg/kg) =
Human dose (mg/kg) × K_m ratio (Eq. 2)

Experimental design Phase 2: Oral glucose tolerance test (OGTT) using RS and LRS on type 1 diabetic rats

Diabetes induction

Rats were fasted for 6 h, and had free access to deionised water before Streptozotocin (STZ) injection. STZ (Merck, Germany) was freshly prepared by dissolving STZ in 0.1 mM cold citrate buffer (pH: 4.5). Diabetes mellitus (DM) was induced by a single dose through intraperitoneal injection of STZ (50 mg/kg) into fasted rats (Furman, 2015). Blood glucose levels were evaluated 3 d later by tail prick using a calibrated hand-held glucometer. Rats with blood glucose levels above 11.1 mmol/L were considered diabetic (Furman, 2015).

Oral glucose tolerance test (OGTT) using RS and LRS on diabetic rats

A glucose oral tolerance test was conducted on the 48 fasted diabetic rats. The rats were distributed into eight groups, with six rats per group. As described previously, rats were given different solutions based on the assigned groups. One group was added, which received metformin (dissolved in distilled water) at a dose of 150 mg/kg (MET) (Za'abi *et al*., 2021). Glucose levels were measured before the administration of different solutions (0 min) and time points of 15, 30, 60, 90, and 120 min after the administration. Glycaemia was determined at each time point using a calibrated hand-held glucometer (Accu-Chek Performa, Roche, Mannheim, Germany) (St-Pierre *et al*., 2014).

Experimental design Phase 3: Acute exposure to different doses of RS and LRS

After 2 d of glucose tolerance test on diabetic rats, rats received a daily gavage from either LRS, RS, or metformin for 2 w as follows:

i. Group 1 (Diabetic control, DB): Normal diet + distilled water.
ii. Group 2 (Normal control, CT): Normal diet + distilled water.
iii. Group 3 (LRS-L): Normal diet + LRS low dose (2.6 g/kg).
iv. Group 4 (LRS-M): Normal diet + LRS medium dose (5.2 g/kg).
v. Group 5 (LRS-H): Normal diet + LRS high dose (6.9 g/kg).
vi. Group 6 (RS-L): Normal diet + RS low dose (2.6 g/kg).
vii. Group 7 (RS-M): Normal diet + RS medium dose (5.2 g/kg).
viii. Group 8 (RS-H): Normal diet + RS high dose (6.9 g/kg).

After 2 w of treatment, animals were fasted overnight and anaesthetised with an overdose of ketamine-xylazine (50:10 mg/kg, intraperitoneal injection), and then sacrificed by cardiac puncture. Blood was drawn in ordinary serum tubes, and immediately centrifuged to collect serum. Samples were kept at -80°C for further analysis.

Biochemical analysis

Fasting insulin levels were measured using ultrasensitive rat insulin (80-INSRTU-E 10) enzyme-linked immunosorbent assay (ELISA) kits (Alpco, Salem, NH, USA). In addition, fasting serum glucose, total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein were measured using an automatic biochemical analyser (Hitachi 902, Roche, Germany). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated based on fasting insulin (µU/mL) and glucose levels (mmol/L) (Er *et al*., 2016) using Eq. 3:

HOMA-IR = insulin level (µU/mL) × glucose levels (mmol/L)/22.5 (Eq. 3)

Statistical analysis

The data were presented as mean ± standard deviation (SD). Statistical analysis between the samples was performed using One-way ANOVA and followed by Dunnett's *post hoc* multiple comparison tests using Minitab 18.1 software. Differences in the means were considered statistically significant at $p < 0.05$. All experimental data values were plotted using the GraphPad PRISM program version 9 for Windows. The areas under the curve (AUC) for glucose were calculated using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA), using the value at t = 0 min of each group as the baseline.

Results

Postprandial glycaemic response in normal rats (Phase 1)

An oral glucose tolerance test (OGTT) using three different doses of LRS and RS (Low, L; Medium, M; and High, H) was conducted on fasted healthy rats (Figure 1). On glycaemic response for post-LRS-L administration, glucose level increased significantly ($p < 0.05$) at 15, 30, and 60 min from its initial glucose level of 4.44 ± 0.54 mmol/L. However, the glucose level decreased to 5.28 mmol/L at 120 min, but was not significantly different from the initial glucose level, and continued to decrease until it reached 4.50 ± 0.68 mmol/L at 180 min. Compared with LRS-L ingestion, RS-L and glucose led to a more significant increase in glycaemia than LRS-L, since the glucose level was significantly higher than the initial glucose level at 15, 30, 60, and 120 min. The decrease in glycaemia post-RS-L ingestion only occurred at 180 min (4.96 ± 0.88 mmol/L), which was not significantly different from the initial glucose level. It was observed that glucose level peaked instantly at 15 min after RS-L ingestion. The total increment of glucose level at 15 min was 71.02% higher compared to 0 min. The glucose level peaked at 30 min after LRS ingestion, and the percentage of increase was 58.11% at 30 min.

Post-gavage LRS-M showed higher glycaemia compared to LRS-L. LRS-M oral administration showed less glycaemia than RS-M, where the glucose level decrease to the initial level at 120 min (5.10 ± 0.79 mmol/L) after LRS-M ingestion, and was not significantly different from the initial glucose level. However, after RS-M ingestion, glucose levels were significantly higher at 15, 30, 60, 120, and 180 min compared to the initial glucose level. Glucose peak after LRS-M and RS-M ingestion occurred similarly at 15 min. However, the glucose increase was lower after LRS-M than RS-M ingestion (55.19 and 81.99%, respectively).

At high doses of LRS and RS (LRS-H and RS-H), glucose level decreased to the initial glucose level at 120 min after LRS-H ingestion, while post-RS-H ingestion showed persistent hyperglycaemia until 180 min. The highest glucose increments after LRS-H and RS-H ingestion occurred at 15 min, and the glucose increment was higher after RS-H ingestion than LRS-H (81.86 and 73.09%, respectively).

Furthermore, the total glycaemic increments induced by different doses of LRS or RS, as revealed by the calculation of AUC (Figure 2), showed that the global glucose response to different doses of RS was higher than LRS, but not significant. On the contrary, LRS-L had the slightest glucose global response among different doses of LRS.

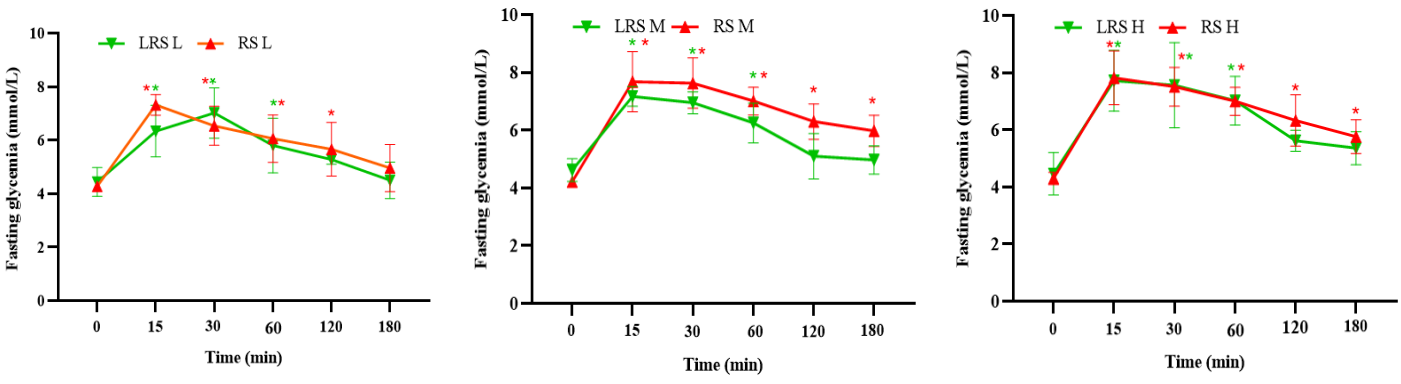

Figure 1. Glycaemic responses following ingestion of LRS or RS at different doses on Sprague-Dawley normal rats: **(A)** glycaemia following ingestion of low dose of LRS or RS; **(B)** glycaemia following ingestion of medium dose of LRS or RS; and **(C)** glycaemia following ingestion of high dose of LRS or RS. Data are mean ± SD of six replicates (n = 6 rats/group). *Significantly different from 0 h. LRS-L: less refined sugar, low dose; LRS-M: less refined sugar, medium dose; LRS-H: less refined sugar, high dose; RS-L: refined sugar, low dose; RS-M: refined sugar, medium dose; and RS-H: refined sugar, high dose.

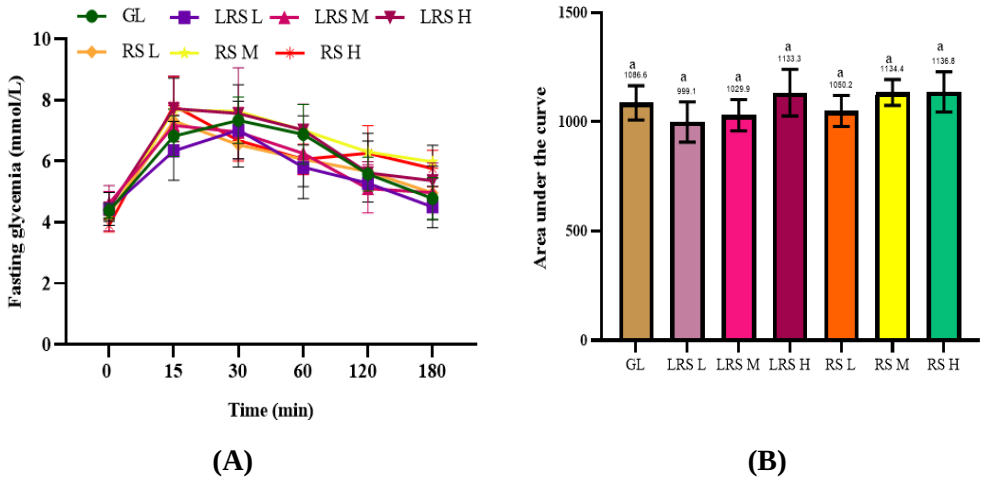

Figure 2. Glycaemic responses following ingestion of different types of sugar on Sprague-Dawley normal rats: **(A)** glycaemic response of different types of sugar and their different doses; **(B)** in the area under the glycaemic response curve presented in Figure A, groups sharing the same letter are not significantly different from the GL reference solution. Data are mean ± SD of six replicates (*n* = 6 rats/group). GL: glucose reference solution; LRS-L: less refined sugar, low dose; LRS-M: less refined sugar, medium dose; LRS-H: less refined sugar, high dose; RS-L: refined sugar, low dose; RS-M: refined sugar, medium dose; and RS-H: refined sugar, high dose.

Postprandial glycaemic response in diabetic rats (Phase 2)

An oral tolerance test using different doses of LRS and RS was conducted on STZ-induced diabetic rats, and the glycaemic response was compared to the standard solution (glucose) and a reference drug, metformin (Figure 3). The results showed that there was a significant decrease in blood glucose level in the group that received the reference drug metformin from 17.47 ± 1.36 mmol/L (at 0 min) to 5.83 ± 1.19 mmol/L (at 180 min), representing a 66.63% decrease. On the other hand, different types of sugars in all doses increased the glucose level until 180 min compared to the pre-prandial glucose level, except for the group that received LRS-M.

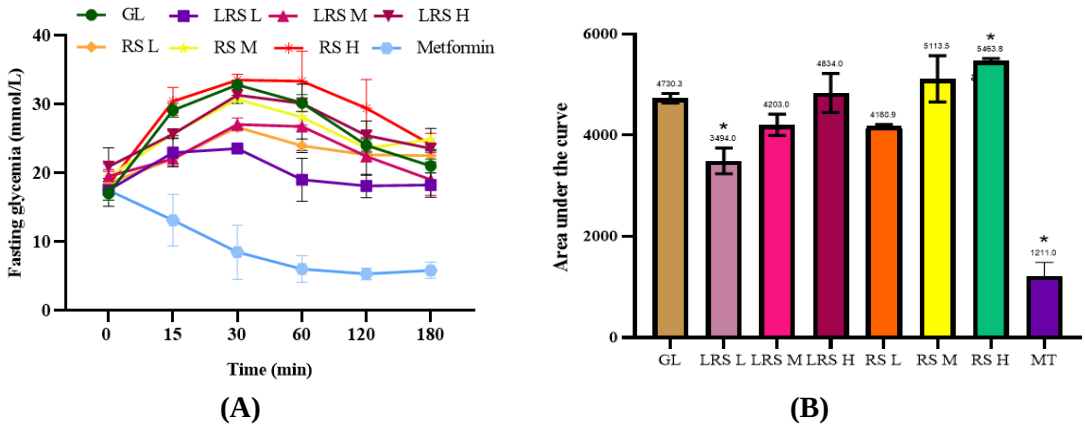

Figure 3. Glycaemic responses following ingestion of different types of sugar on STZ-induced diabetic rats: **(A)** glycaemic response of different types of sugar at different doses. **(B)** in the area under the glycaemic response curve presented in Figure A, groups sharing the same letter are not significantly different from the GL reference solution. Data are mean ± SD of six replicates (*n* = 6 rats/group). GL: glucose reference solution; LRS-L: less refined sugar, low dose; LRS-M: less refined sugar, medium dose; LRS-H: less refined sugar, high dose; RS-L: refined sugar, low dose; RS-M: refined sugar, medium dose; RS-H: refined sugar, high dose; and Met: metformin.

Following LRS-load at a low dose, glucose level increased steadily, but there was no significant increase during the entire period compared to 0 min (Figure 4). RS- and glucose-load at low doses caused significantly higher glycaemia until 180 min compared to 0 min ($p < 0.05$). LRS-M load showed significantly higher glucose levels at 30 and 60 min. However, the sugar level dropped at 120 and 180 min, and was not significantly different from 0 min. A slight decrease in glucose level was noticed at 180 min (-2.56%) after LRS-M load compared to pre-prandial glucose level, but not significant. Compared to LRS-M, RS-M led to significantly higher glycaemia at 30, 60, and 120 min. Similarly, RS at a high dose resulted in significantly higher glycaemia over the entire period of 180 min, while LRS-H showed significant glycaemia only at 30 and 60 min. The increment percentage of glucose level at 180 min compared to pre-prandial glucose level was noticed to be lower for LRS-L, LRS-M, and LRS-H (4.28, -2.56, and 3.11%, respectively) by comparison with RS-L, RS-M, and RS-H (21.62, 18.86, and 31.97%, respectively).

The AUC values of the glycaemic response in diabetic rats are shown in Figure 3. The metformin group's global glucose response was significantly lower, followed by LRS-L.

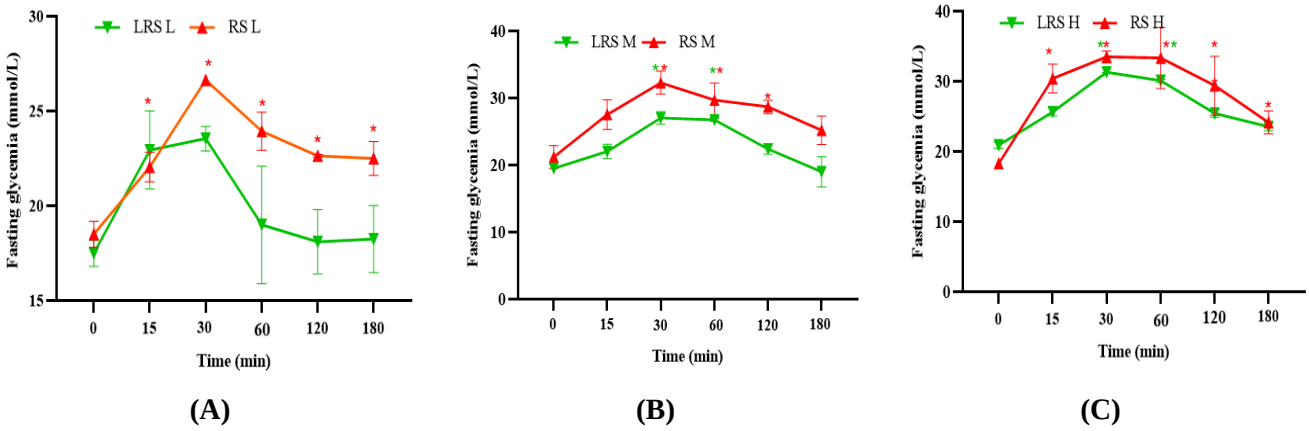

Figure 4. Glycaemic responses following ingestion of LRS or RS at different doses on STZ-induced diabetic rats: **(A)** glycaemia following ingestion of low dose of LRS or RS; **(B)** glycaemia following ingestion of medium dose of LRS or RS; and **(C)** glycaemia following ingestion of high dose of LRS or RS. Data are mean ± SD of six replicates (n = 6 rats/group). *Significantly different from 0 h. LRS-L: less refined sugar, low dose; LRS-M: less refined sugar, medium dose; LRS-H: less refined sugar, high dose; RS-L: refined sugar, low dose; RS-M: refined sugar, medium dose; and RS-H: refined sugar, high dose.

Metabolic effects after acute exposure to LRS or RS in diabetic rats (Phase 3)

The data on glucose, insulin, and lipid profiles are presented in Tables 1 and 2, respectively. After 2 w intervention, glucose levels significantly differed between the treatment groups. Diabetic groups yielded significantly higher serum glucose levels than the normal group. Serum glucose level was found to be lower but not significant ($p > 0.05$) for groups receiving LRS-L, LRS-M, and LRS-H (11.53 ± 2.51, 12.68 ± 5.09, and 14.88 ± 1.46 mmol/L, respectively) in comparison with corresponding doses of RS-L, RS-M, and RS-H (12.48 ± 0.74, 15.02 ± 1.28, and 15.70 ± 0.05 mmol/L, respectively). Consumption of high doses of either RS or LRS showed the highest fasting glucose level compared to other doses (low and medium).

Concerning insulin level and lipid profile, all diabetic groups had significantly lower insulin secretion compared to the normal group. No significant difference was observed in insulin levels between LRS and RS-treated groups. However, consumption of LRS showed lower insulin resistance, as revealed by decreased fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR) compared to RS treatment groups.

Table 1. Glycaemic profile after two weeks of treatment on STZ-induced diabetic rats.

Treatment	Glucose		Insulin		Insulin resistance	
group	(mmol/L)	95% IC	(µU/L)	95% IC	HOMA-IR	95% IC
NG	6.50 ± 0.50[a]	(3.72, 9.28)	15.10 ± 0.83[b]	(13.19,17.01)	4.36 ± 0.03[a]	(2.59, 6.13)
DB	14.11 ± 1.99[b]	(11.33,16.89)	11.11 ± 1.04[a]	(9.2, 13.02)	6.91 ± 0.68[a]	(5.14, 8.68)
MT	14.62 ± 1.15[b]	(11.84,17.40)	10.25 ± 0.30[a]	(8.34, 12.16)	6.65 ± 0.45[a]	(4.88, 8.42)
LRS-L	11.53 ± 2.51[b]	(8.75, 14.31)	6.84 ± 2.14[a]	(4.93, 8.75)	3.61 ± 1.54[a]	(1.84, 5.38)
LRS-M	12.68 ± 5.09[b]	(9.89, 15.46)	10.60 ± 2.12[a]	(8.69, 12.51)	5.70 ± 1.67[a]	(3.93, 7.47)
LRS-H	14.88 ± 1.46[b]	(12.09,17.66)	8.79 ± 0.66[a]	(6.88, 10.70)	5.83 ± 0.81[a]	(4.06, 7.60)
RS-L	12.48 ± 0.74[b]	(10.76,14.20)	10.18 ± 1.72[a]	(7.85, 12.51)	5.66 ± 1.16[a]	(3.89, 5.66)
RS-M	15.02 ± 1.28[b]	(13.33,16.74)	10.14 ± 3.53[a]	(8.23, 12.05)	6.65 ± 1.88[a]	(4.88, 8.42)
RS-H	15.70 ± 0.05[b]	(13.98,17.42)	10.53 ± 4.89[a]	(8.20, 12.86)	7.36 ± 3.41[b]	(5.59, 9.13)
p-value	< .01		< .001		< .01	
η^2	0.707		0.913		0.429	

Data are mean ± SD of six replicates (*n* = 6 rats/group). Groups with different lowercase letters are significantly different from normal control group. NG: normal group; DB: diabetic group; MT: metformin; LRS-L: less refined sugar, low dose; LRS-M: less refined sugar, medium dose; LRS-H: less refined sugar, high dose; RS-L: refined sugar, low dose; RS-M: refined sugar, medium dose; RS-H: refined sugar, high dose; 95% IC: confidence interval; and η^2: Eta squared (effect size).

Table 2. Lipid profile after two weeks of treatment on STZ-induced diabetic rats.

Treatment	HDL		LDL		Cholesterol		Triglyceride	
group	(mmol/L)	95% IC	(mmol/L)	95% IC	(mmol/L)	95% IC	(mmol/L)	95% IC
NG	0.82 ± 0.15[a]	(0.69, 0.96)	0.18 ± 0.04[a]	(0.12, 0.25)	1.21 ± 0.10[a]	(0.95, 1.47)	0.57 ± 0.08[a]	(0.26, 0.88)
DB	0.47 ± 0.08[b]	(0.35, 0.63)	0.16 ± 0.03[a]	(0.08, 0.21)	1.14 ± 0.31[a]	(0.88, 1.40)	0.67 ± 0.06[a]	(0.36, 0.98)
MT	0.64 ± 0.04[a]	(0.48, 0.79)	0.24 ± 0.02[a]	(0.19, 0.31)	1.19 ± 0.09[a]	(0.93, 1.45)	0.48 ± 0.02[a]	(0.17, 0.79)
LRS-L	0.66 ± 0.04[a]	(0.49, 0.81)	0.17 ± 0.08[a]	(0.12, 0.25)	1.16 ± 0.14[a]	(0.89, 1.42)	0.48 ± 0.15[a]	(0.17, 0.79)
LRS-M	0.69 ± 0.10[a]	(0.54, 0.86)	0.23 ± 0.08[a]	(0.16, 0.30)	1.33 ± 0.14[a]	(1.97, 1.59)	0.61 ± 0.04[a]	(0.29, 0.92)
LRS-H	0.51 ± 0.10[b]	(0.38, 0.65)	0.24 ± 0.05[a]	(0.19, 0.31)	1.14 ± 0.18[a]	(0.88, 1.40)	0.38 ± 0.09[a]	(0.07, 0.69)
RS-L	0.41 ± 0.17[b]	(0.27, 0.54)	0.22 ± 0.02[a]	(0.15, 0.29)	1.12 ± 0.24[a]	(0.86, 1.38)	0.49 ± 0.09[a]	(0.18, 0.80)
RS-M	0.41 ± 0.07[b]	(0.29, 0.53)	0.20 ± 0.03[a]	(0.13, 0.28)	1.04 ± 0.04[a]	(0.78, 1.29)	0.79 ± 0.32[a]	(0.48, 1.10)
RS-H	0.44 ± 0.11[b]	(0.30, 0.57)	0.27 ± 0.09[a]	(0.15, 0.29)	1.38 ± 00.34[a]	(1.12, 1.64)	0.83 ± 0.48[a]	(0.52, 1.14)
p-value	0.001		> .05		> .05		> .05	
η^2	0.6169		0.267		0.399		0.503	

Data are mean ± SD of six replicates (*n* = 6 rats/group). Groups with different lowercase letters are significantly different from normal control group. NG: normal group; DB: diabetic group; MT: metformin; LRS-L: less refined sugar, low dose; LRS-M: less refined sugar, medium dose; LRS-H: less refined sugar, high dose; RS-L: refined sugar, low dose; RS-M: refined sugar, medium dose; RS-H: refined sugar, high dose; HDL: high-density lipoprotein; LDL: low-density lipoprotein; 95% IC: confidence interval; and η^2: Eta squared (effect size).

Besides, HDL levels were significantly higher in serum samples of all groups compared to RS-treated groups. The LDL, cholesterol, and triglyceride levels showed no differences among the groups ($p > 0.05$).

Discussion

Although the detrimental health impact of RS has been studied extensively, only a few investigations have addressed the potential beneficial effects of sugary products that are less refined from natural sugar. LRS is a product from food-grade sugar mills, and prepared through steps such as washing, extraction, minimal refining, crystallisation, drying, and packaging (Azlan *et al*., 2023). Compared to regular BS, LRS has a milder flavour and a lighter colour. LRS is a unique sugar due to being clump-free, with free-flowing texture and taste. The composition of LRS is close to MRBS (Azlan *et al*., 2020), which is high in nutritional value, especially antioxidant (Azlan *et al*., 2023). However, the metabolic effect of this sugar still needs to be investigated in humans and animals (Zidan and Azlan, 2022; Ebadi and Azlan, 2023). The present work compared the glycaemic response of LRS and RS in normal and type 1 diabetic rats. In addition to the glycaemic response, the metabolic response was also evaluated after two weeks of intervention, for the two types of sugar in type 1 diabetic rats.

The present work showed that RS and LRS impacted glucose regulation differently in normal and diabetic rats. Indeed, the peak of glycaemia and global glucose response was lower after LRS oral administration than RS or reference solution (glucose) in normal and type 1 diabetic rats. Firstly, Sprague-Dawley healthy rats administered with any of the three LRS doses had reduced blood sugar levels compared to the control group (administrated with glucose) or RS groups. While the mean glucose level was only significantly elevated for the first hour post-treatment in the LRS groups, the mean glucose levels in both RS-M and RS-H groups remained significantly higher for up to three hours. The variance in glycaemic response induced by the different types of sugar might be linked to their different composition of carbohydrates and other micronutrients.

It was reported that the primary differences between unrefined sugar and RS are the higher amounts of sucrose (98.33 to 99.63 g per 100 g) in RS compared to unrefined sugar (88.46 to 89.85 g per 100 g), and the amount of minerals and other minor constituents (Ebadi and Azlan, 2020). LRS is a type of centrifugal sugar with minor components such as phenolics, selenium, and potassium, similar to minimally processed BS (Azlan *et al*., 2020). Generally, unrefined sugar has a superior nutritional value and phytochemical content compared to RS due to the presence of molasses and less refining processes (Zidan and Azlan, 2022). Eggleston (2018) reported that molasses contains about 3,000 mg/L of total phenolic content, a near equivalent to pomegranate juice (2,850 mg/L). Various quantities of polyphenols were detected and reported in some unrefined sugar and LRS samples (Iqbal *et al*., 2017; Azlan *et al*., 2020; 2023). In particular, LRS was recently reported to contain higher polyphenols of 57.72 μg/g, compared to 22.06 μg/g in RS (Azlan *et al*., 2023). The major phenolic compounds detected in LRS include *p*-coumaric, caffeic, syringic, and ferulic acids. At the same time, tricin, apigenin, luteolin, and vanillin were also the main flavonoids in LRS (Azlan *et al*., 2023). Polyphenolic compounds in the food matrix may influence carbohydrate digestion, absorption, and metabolism.

Polyphenolic may influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion enzymes, inhibition of glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic cells, modulation of glucose release from the liver, activation of insulin receptors, and glucose uptake in insulin-sensitive tissues (Hanhineva *et al*., 2010; Kim *et al*., 2016). α-Amylase and α-glucosidase are the key enzymes responsible for the digestion of dietary carbohydrates to free glucose (Kim *et al*., 2016). The free glucose is absorbed across the intestinal enterocytes *via* specific transporters. The inhibition of the digestive enzymes or glucose transporters would reduce the rate of glucose release and absorption in the small intestine, and consequently suppress postprandial hyperglycaemia (Kim *et al*., 2016). In a recent investigation, LRS showed minor inhibition of α-amylase, and a moderate inhibition of α-glucosidase activities (Azlan *et al*., 2023). Ranilla *et al*. (2008) reported that different varieties of unrefined sugar (containing phenolic compounds) showed α-glucosidase inhibitory properties in the range of 25 to 50%, correlating with high phenolic content and radical scavenging capacity. Therefore,

low postprandial hyperglycaemia observed after LRS dose consumption can be due to their inhibition of α-glucosidase and α-amylase activities.

Additionally, intestinal glucose absorption is mediated by active transport *via* the Na^+-dependent glucose transporter SGLT1, and facilitated by sodium-independent transport *via* the glucose transporter GLUT2 (Hanhineva *et al*., 2010). The NA^+-dependent glucose transporter SGLT1 was inhibited by ferulic and caffeic acids (Welsch *et al*., 1989), while the glucose transport, GLUT2, was inhibited by apigenin (Johnston *et al*., 2005). As reported recently, both polyphenols were detected in LRS (Azlan *et al*., 2023). Therefore, these polyphenolic compounds in LRS might have resulted in a lower glucose absorption rate in the intestine, resulting in lower glucose levels throughout the period. However, more studies are needed to assess whether unrefined sugar and LRS can impact glucose transporters in the small intestine.

We also studied the impact of different types of sugar at different doses on STZ-induced diabetic rats. To our knowledge, this is the first work evaluating LRS's glycaemic response on STZ-induced diabetic rats. The present work found that postprandial glycaemia was attenuated after LRS dose administration compared to RS on STZ-induced diabetic rats. LRS-L administration showed no significant increase in mean glucose level over 180 min. The incremental area under the LRS-L glucose response curve was also significantly lower than the GL control group. Although LRS-M significantly increased glycaemia until 60 min, and at 180 min, it was the only group that showed a minor decrease (2.56%) but not significant in mean glucose level compared to pre-prandial glucose level. However, this decrease did not affect the incremental area under the glucose response curve, where the AUC of LRS-M was not significantly different compared to the control group. The most likely explanation of the decrease at 180 min is that LRS at a medium dose may possess an adequate amount of bioactive molecules that attenuate the glycaemic response compared to LRS-L, which probably caused a slight decrease in glycaemia at 180 min. Adam *et al*. (2010) reported that the less amount of bioactive constituent(s) present in low doses of plant extract, the longer time it takes bioactive constituent(s) in the extract to enter circulation, and reach the target tissue to regulate glucose metabolism. In the present work, we also investigated the effect of metformin (first-line antidiabetic medication) on STZ-induced diabetic rats. The metformin group showed significantly lower glycaemia at 30 min, and the total glycaemic decrease accounted for 66.63% at 180 min, the lowest among all the other groups. Metformin is generally the most widely used antidiabetic medication, and is a so-called (insulin) sensitiser (Röder *et al*., 2016). It not only diminishes hepatic glucose output due to glycogenolysis/gluconeogenesis, but also enhances glucose uptake into peripheral tissues, such as skeletal muscle, by activating 5′-adenosine monophosphate-activated protein kinase (AMPK-α2) (Röder *et al*., 2016). The variation in glycaemic decrease between metformin and our tested sugar was expected, since both RS and unrefined sugar have almost similar and high carbohydrate content.

Nevertheless, they differ regarding nutrients such as polyphenols and minerals, which could have influenced the digestion and absorption of the two types of sugar, and consequently impacted glucose metabolism. Although both sugars did not show a significant glucose decrease compared to metformin, LRS roughly showed better glycaemic control over 180 min compared to RS. This agreed with other reports, where administration of unrefined natural sweetener resulted in lower glycaemia compared to RS in diabetic subjects (Nagai *et al*., 2013; 2015). Minimally refined sugar was also reported to have a low GI (< 55), and thus was categorised as low GI food. In contrast, RS has a higher glycaemic index (< 60), and categorised as medium GI food (Azlan *et al*., 2022).

Indeed, a sweetener that can achieve a low glycaemic profile is of great interest for healthy or diabetic subjects. For instance, foods and beverages that cause low glycaemic response could improve insulin sensitivity because they gradually increase the plasma glucose, and then slow down the secretion of insulin, may reduce insulin demand, and decrease β-cell dysfunction (Radulian *et al*., 2009). Consequently, the population may have better blood glucose control, and maintain ideal metabolic health. On the contrary, foods that cause higher glycaemia have been shown to trigger a rapid increase in postprandial blood sugar, induce insulin resistance, and cause pancreatic exhaustion, and consequently increase the risk of type 2 diabetes mellitus and cardiovascular disease in the Western population, as well as in Asian population (Pavithran *et al*., 2020). For diabetic patients, the primary goal in managing diabetes is to achieve as near normal blood glucose

regulation (postprandial and fasting) as possible. The total amount and type of carbohydrates (low or high GI) consumed have the highest influence on glycaemic response (ADA, 2019). According to the American Diabetes Association (ADA, 2019), people with diabetes and those at risk are advised to avoid sugar-sweetened beverages.

Nevertheless, based on a study by Murad *et al*. (2014), in the assessment of diet status in diabetic patients, it was found that only 27.5% of diabetic patients did not consume sugary foods and sweets. This might be because two-thirds of packaged foods contain added sugars (*e.g*., sucrose, glucose, or fructose) to enhance flavour or prolong shelf life (Valle *et al*., 2020). Therefore, based on both OGTT experiments in the present work, it was evident that LRS induced lower glycaemia, and extended for a short period at all doses, compared to corresponding doses of RS, in diabetic and normal rats, especially the LRS-L or LRS-M, owing to its bioactive profile of some insulin-mimetic minerals (Azlan *et al*., 2020). LRS can be suggested as a good substitute for patients with diabetes. However, additional long-term studies in animal and human trials are required to determine the biological role of LRS in diabetes management and its complications in the coming time.

The efficacy of LRS consumption was also investigated in the present work for the first time compared to RS on metabolic response (glucose, insulin, and lipid profile) in diabetic rats. Diabetes mellitus is a metabolic disease characterised by sustained high glucose levels and disruption of carbohydrate, fat, and protein metabolism associated with an absolute or relative deficiency in insulin secretion or action (Hassan *et al*., 2019). Hyperglycaemia, hyperlipidaemia, hypertension, atherosclerosis, retinopathy, neuropathy, and nephropathy are the major diabetes complications (Hassan *et al*., 2019). Experimentally, STZ-induced cytotoxicity in pancreatic cells is mediated by oxidative stress, alterations in cellular metabolism, and mitochondrial dysfunction, leading to the induction of diabetes in animals (Al Nahdi *et al*., 2017).

In the present work, fasting glucose level significantly increased in the diabetic groups, and was associated with a significant decrease in insulin level compared to the normal group. These results could have been due to the cytotoxic effect of STZ, which caused pancreatic cell destruction and diminished insulin secretion in diabetic rats compared to the normal rats (Al Nahdi *et al*., 2017). Besides, fasting serum glucose level, insulin level, and HOMA-IR score were lower in LRS-treated groups than in RS-treated groups. Similar short-term studies confirmed that natural sweeteners consumption, such as honey (Erejuwa *et al*., 2010; Hassan *et al*., 2019) and xylitol (Rahman and Islam, 2014), resulted in lower glucose levels in diabetic rats, which was consistent with our findings. Less adverse effect on insulin resistance and glycaemia was also reported following BS or honey consumption for four months compared to RS in healthy rats, on a normal or high-fat diet (Sánchez-Tapia *et al*., 2019; 2020).

While polyphenols might play a significant role in regulating glucose metabolism in healthy and diabetic subjects, as discussed earlier, unrefined sugar and LRS contain an adequate amount of certain minerals such as selenium, potassium, magnesium, calcium, and chromium (Azlan *et al*., 2020), which also have a significant biological role in regulating glucose metabolism (Zidan and Azlan, 2022). MRBS has been shown to contain higher selenium content than BS or white sugar (Azlan *et al*., 2023). Based on our recent investigation, LRS demonstrated superior mineral content, particularly in selenium, potassium, and magnesium, compared to RS. The presence of selenium makes LRS an ideal healthier choice of sweetener for healthy and type 2 diabetic patients (Azlan *et al*., 2023). Selenium is an antioxidant and insulin-mimetic nutrient (Fontenelle *et al*., 2018). It is essential for the activity of selenoproteins, such as glutathione peroxidase 1 (GPx1) and selenoprotein P (SelP), which have been reported to associate with glucose homeostasis, especially in maintaining a redox balance to promote the normal synthesis and secretion of insulin (Fontenelle *et al*., 2018). SelP is highly expressed in pancreatic islets, which act as antioxidants to protect β cells (Zhao *et al*., 2022). GPx1 can degrade intracellular H_2O_2. In pancreatic islets, GPx1 reduces the damage of H_2O_2 on β cells, and promotes the normal secretion of insulin (Zhao *et al*., 2022). Biologically, mammals have a limited reservoir of selenium, thus need a regular supply through diet and water (Dubey *et al*., 2020). Iqbal *et al*. (2017) reported that raw, unrefined sugar contains 74% more chromium than RS. Chromium improves insulin binding, receptor number, and receptor enzymes by increasing insulin sensitivity, β-cell sensitivity, and insulin internalisation (Dubey *et al*., 2020). Thus, LRS should be considered more than a

sugar source, since it maintains lower glycaemia, and might provide the host body with beneficial nutrients such as polyphenols and minerals.

Although insulin resistance is generally considered to be a distinctive feature of type 2 diabetes mellitus, RS-H consumption resulted in significant insulin resistance as compared to other groups. Wolosowicz *et al.* (2020) reported that excessive adipose tissue mass, exorbitant free fatty acids level, chronic hyperglycaemia, reactive oxygen species production, and chronic inflammation were all significant factors that contributed to the development of insulin resistance in type 1 diabetes mellitus. RS consumption was associated with developing one or more of the risk factors of insulin resistance in type 1 diabetes mellitus. For example, RS consumption has been linked to increase in adiposity and fat mass even with the absence of HFD (Sánchez-Tapia *et al*., 2019; 2020; Shamsi-Goushki *et al*., 2021), decrease the membrane translocation of GLUT4 and adipocyte glucose uptake (Sánchez-Tapia *et al*., 2019), promote the expression of GLUT2 in the enterocytes resulting in increased glucose transport (Sánchez-Tapia *et al*., 2019), and recruit macrophages and promote inflammation (Sánchez-Tapia *et al*., 2019; 2020; Shamsi-Goushki *et al*., 2021), in which all could lead to the development of insulin resistance. However, in the present work, the difference between RS and LRS on lipid profile has not been significantly affected, possibly due to the short exposure period. Therefore, longer exposure time is required to confirm their effect on lipid profile.

Based on the data collected from OGTT on normal and STZ-induced diabetic rats, and metabolic profile assessment after short-term exposure to diabetic rats, it can be concluded that consumption of LRS at high dose resulted in the highest glucose response and insulin resistance compared to the other two doses of the same sugar in normal or diabetic rats; RS consumption at high dose also showed similar trend. We thus recommend limiting the LRS and RS intake following WHO recommendations at 5 and 10% of total calories to maintain a balanced glycaemic profile. Additionally, the lower glycaemic profile observed after LRS consumption suggested that daily substituting RS with LRS is favourable for glucose level management. The present work is the first to determine the glycaemic response of LRS compared to other sugars in diabetic and normal rats. While the present work provided valuable insights, it was limited by its short duration. Therefore, the long-term effects of the consumption or replacement of LRS with RS must be determined. Future long-term studies are necessary to evaluate LRS's efficacy on glycaemic control, endogenous antioxidant enzymes, oxidative stress, and inflammation, and to establish the exact mechanism(s) of action. The long-term study is underway, and hopefully will provide answers for a better understanding of the health benefits of LRS.

Conclusion

Our results showed that LRS at different doses had better glycaemic profiles than RS in normal and type 1 diabetic rats. LRS could be a viable and healthier substitute for RS, and should be considered in dietary recommendations. However, detailed, long-term, and regulated studies are essential and underway to enhance the outcomes, and elucidate the mechanism underlying the potential role of LRS in diabetes management.

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