Macronutrient Content of Chocolate Muffin Using Different Sweeteners and Their Effects on Metabolic Rate

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Abstract:

Background: Excessive sugar intake affects the quality of nutritional status and overall health issues among people globally. Thus, reduction in the amount of sugar consumption using low-calorie sweeteners (LCS) in foods and beverages have become a key focus for a healthier diet among consumers. However, recent studies assessing the disadvantages of the LCS have shown that consumers tend to compensate for the diluted energy content by eating more solid food calorie at subsequent meals. This study aimed to assess the differences in macronutrient content of chocolate muffin when sugar is substituted with LCS (stevia and aspartame) and their effect on metabolic rate of adults.

Materials and Methods: Three different chocolate muffins containing 3 different sweeteners (sugar, stevia, and aspartame) were prepared. The test food was used for macronutrient analysis and 28 participants were recruited into the study where Resting Metabolic Rate (RMR) data, pre and post consumption of the test foods was measured.

Result: Macronutrient analysis showed that sugar muffin came out highest in energy content (299 kcal) followed by stevia and aspartame with 248 kcal each. However, there were no significant changes in metabolic rate with ingestion of LCS in our chocolate muffin at all time intervals.

Conclusion: Based on the hypothesis and the end results, stevia and aspartame's consumption does not significantly reduce the metabolic rate that can subsequently reduce total energy expenditure.

Keywords: Low-calorie sweeteners; metabolic rate; sugar; stevia; aspartame

Introduction:

Today, intervention studies investigating the effect of low-calorie sweeteners (LCS) consumption have shown potential benefits towards human body weight management compared to daily table sugar. LCS is commonly known as sugar substitutes due to their potent sweet taste intensities with minimal amount of calorie and no calorie (for some of the LCS). When used in beverages and food products, LCS do not increase the blood sugar levels even after consumption. Due to this characteristic of potent sweetness intensity, only a small amount of a sweetener is needed to replace the sweetness of a much larger amount of sugar. Thanks to that, the demand for low-calorie based products and beverages allows consumers to eat foods without the risk of consuming additional calories as provided by sugar-based products (Shankar et al., 2013). This makes LCS containing foods a preferable choice for consumers especially for diabetics and obese patients considering that LCS is neither carbohydrate, nor fat and do not fit in any of the other categories of the diabetic exchange.

There has been much debate related to the pros and cons of LCS towards human health since their introduction into the public market in the 50s and 60s. One of the popular health issues that lead to the ban of LCS by the FDA was the commonly used LCS; cyclamate which was shown to have carcinogenic effects. Since then, the LCS industry has succeeded in producing safe LCS products for human consumption. However, there still lacks sufficient scientific evidence on the mechanism of action and safety of LCS. Table 1 shows the most used LCS that have been tested and approved and generally recognized as safe (GRAS) by the U.S Food and Drug Administration (FDA).

Table 1 Properties of low-calorie sweeteners

Low-Calorie Sweetener, LCS	Sweetness compared to sugar (sucrose)	Acceptable Daily Intake, ADI[a]	Brand name
Acesulfame K	200 times sweeter than sugar[b]	15[b]	Sunnet®, Sweet One®
Saccharin	200 times sweeter than sugar[b]	2.5[b]	Sweet'N Low®, SweetTwin®, Nectar Sweet®
Sucralose	600 times sweeter than sugar[b]	15[b]	Splenda®
Aspartame	180 – 200 times sweeter than sugar[b]	50[b]	Equal®, NutraSweet®, SugarTwin®
Neotame	7,000 – 13,000 times sweeter than sugar[b]	18[b]	Newtame®
Stevia	200 – 400 times sweeter than sugar	4	Equal®, Truvia®, Purevia®

* Stevia; Rebaudioside A or Reb-A is generally recognized as safe (GRAS) by FDA.
[a]ADI (Acceptable Daily Intake; mg of sweetener/ kg of Human body weight per day)
[b] (Chattopadhyay et al., 2014)

Mechanism of action of sugar and LCS

Sugar is chemically related to sweet-taste substances which consist of sucrose, lactose, and fructose. Sucrose is a carbohydrate from the disaccharide group which consists of a glucose unit and a fructose unit bonded with alpha glycoside bonds. In the human digestive system, alpha-glycosyl bonds are broken down to glucose and fructose by sucrase (digestive enzyme) that is secreted in the small intestine and then absorbed through the small intestines and entire body tissues (Tortora & Derrickson, 2008). Sources of sucrose are derived from either sugar cane or sugar beets. Usually, natural sugar in carbohydrate containing foods such as grains and fruits provide us energy to our cells during the slow process of digesting these foods. However, most processed foods contains added sugar adding to the sugar content that naturally exists in foods containing carbohydrates. Sucrose is one of the main reasons of obesity which promotes metabolic syndrome and diabetes (Howard & Wylie-Rosett, 2002). Thereby, most people attempt to comply with a dietary recommendation and then resort to low calorie-based food products and beverages.

Even with the chemical diversity of natural and synthetic compounds, LCS; aspartame, saccharin, sucralose acesulfame K and stevia all provide a sweet taste to people. Sweetness sensors in our taste buds are triggered when sucrose hits our tongue and then sends a "sweet" signal to our brain. Therefore, any molecules with the same characteristic that can trigger the taste buds will cause the same signals to our brain. Thus, artificial sweeteners can also trigger our taste buds similarly to sucrose.

LCS's effects on metabolic rate

Studies on metabolic rate are important to provide more evidence to support or reject several theories associates with LCS or sugar consumption, metabolic changes, and weight gain. Sylvetsky, Blau, and Rother (2016) stated that not all LCS are the same since they are chemically distinct and some LCS such as aspartame metabolize quickly after ingestion, saccharin is absorbed and excreted in the urine unchanged while the majority of sucralose is not absorbed but excreted in the faeces. It is suggested that LCS have different metabolic and health effects due to dissimilar chemical, physical, biological, and pharmacokinetic properties.

Brown, De Banate, and Rother (2010) explain that dissociation of the sensation of sweet taste from caloric intake can encourage our appetite which leads to greater food consumption, weight gain and is associated with lower diet quality in children. Thus, LCS acts as the behavioural mechanism by altering the taste preferences of sweetened food in place of more healthy foods such as fruits and vegetables. However, Sylvetsky, Rother, and Brown (2011) claimed that there is an increase of LCS consumption within all age groups especially among children since the use of LCS is found in many foods and beverages. Children tend to consume a very high amount of LCS proportionate to their body weight per day. The knowledge of consuming a substance lower in energy tends to trigger people to eat more similar to how people tend to overeat foods after given a low-fat food explaining the association between LCS consumption and weight gain. LCS takes a much smaller amount to produce the same level of sweetness in a product due to their sweetening power that is hundreds of times higher than sugar or sucrose. Bellisle & Drewnowski (2007) mentioned that caloric sugars and intense sweeteners have been blamed to cause obesity due to ambiguous psychobiological signals that confuse the body's regulatory mechanisms hence leading individuals to have an uncontrolled appetite and overeating, after consuming non-caloric sweeteners.

Some research suggests that LCS consumption such as sucralose with carbs may cause metabolic dysfunction. Dalenberg et al. (2020) conducted a study recruiting 45 adult subject who were randomly given three different beverages; Sugar sweetened drink, LCS's sweetened drink (sucralose) and a Combo drink that contained sucralose and a carbohydrate known as maltodextrin. Blood tests were conducted to measure glucose intolerance, and the subject's brain responses to the drinks were determined using fMRI. According to Dalenberg et al. (2020), consumption of sucralose in the presence of carbohydrate dysregulates gut brain regulation of glucose metabolism and impairs insulin sensitivity. This metabolic impairment is associated with decreases in neural responses to sugar even though the sweet taste perception is unaffected and the insulin sensitivity is not altered by sucralose or carbohydrate consumption alone. Depending on the findings by various researchers, LCS have their pros and cons. It is critical that research is conducted to provide evidence that will justify the use of low-calorie sweeteners specifically with regards to resting metabolic rate (RMR).

Materials and Methods:

Food formulation

Chocolate muffins were prepared with three (3) different sweeteners which are sugar (sucrose), stevia, and aspartame as shown in Table 2. Muffin batters were prepared with 70g Sugar, 55g Stevia and 55g Aspartame. The batter was poured into the muffin paper cups (size; 4x4 cm) and baked in the preheated oven at 170°C until done. Each baked muffin (sugar, stevia, and aspartame) was packed in separated polypropylene bags and stored in a dry and cool environment prior to analysis (proximate analysis and RMR assessment). Chocolate muffin was limited to 1 serving for each sweetener (sugar, stevia, and aspartame).

Table 2 Formulation of chocolate muffin

Ingredients	Sweetener		
	Sugar	Stevia (Eversweet)	Aspartame (Equal)
1 ¾ cups (180g) of self-rising flour 1 tsp. (4g) of baking soda ¼ cups (40g) of cocoa powder ½ tsp. (2g) of salt 2 eggs (100g) 1 tbsp. of vanilla essence 1 cup (250ml) of low-fat milk ¼ cups (60ml) of canola oil	1 cup (201g)	18 sachets (1g each)	18 sachets (1g each)

*Formulation above yield 12 servings of chocolate muffin

**18g of stevia (Eversweet) or aspartame (Equal) is equal to 201g in terms of sweetness intensity.

Sample size calculation

A sample size of n =35 was calculated using single proportion formula:

Sample size, n = Z2 P (1-P) / d2

Where Z = 1.96, d = 10%

P = 10%, which is the increment in postprandial metabolic rate after consumption of LCS (Mourao, Bressan, Campbell, & Mattes, 2007)

Thus, n = 35 subjects.

However, due to the limitation in recruiting subjects and completing test procedures due to the COVID-19 pandemic, the study only completed 28 assessments out of 35 calculated samples from 16th July 2018 until 27th February 2020. Livingstone et al. (2000) in their review on "Methodological issues in the assessment of satiety", had mentioned that there are several factors that may produce a negative result which is one of it is study sample may have been too small. However, Livingstone et al. (2000) mentioned again in their review that a sample size of less than 20 is still within a normal range in this type of study. Hence, 28 assessment is sufficient yield results.

Proximate analysis

Proximate analysis had been used in determining the macronutrient status such as carbohydrate, protein, fat, moisture and ash inside each chocolate muffin. Carbohydrate, proteins, and fat content from the test foods were determined according to AOAC 1993 (Method of Analysis for Nutrition Labelling), AOAC 991.20 (Kjeldahl method), and AOAC 963.15 respectively. While for moisture and ash, AOAC 931.04 (Air Oven method) and Pearson's Chemical Analysis of Food, 7th Ed, was used. The AOAC 1993 (Method of Analysis for Nutrition Labelling) determined the energy, kcal for each type of food samples.

Metabolic rate assessment

A proper assessment and analysis was done to determine any metabolic rate changes from the consumption of the food samples. Factors including age, muscle mass, body size (weight and height), gender, physical activity, hormonal factors, drugs, and diet will surely affect the metabolic rate reading. However, due to limitation in recruitment and lack of subjects, the researchers were only able to control a few factors including age, BMI, body fat. No subjects with a clinically diagnosed disease or smoking habits were recruited. Factors such as BMI and body fat was considered before and during the RMR assessment. The study also controlled factors such as physical activity and diet plan before RMR assessment which would affect the reading from the metabolic rate.

Study protocol for RMR assessment

After the screening for health status was done, subjects were recruited and required to attend nine non-consecutive days of test, which had been held in the Anthropometry Lab, Department of Nutrition Sciences, Kulliyyah of Allied Health Sciences, IIUM Kuantan. Subjects were required to refrain themselves from any vigorous physical activities and to fast for at least 8 hours upon arriving on test days. The assessment was conducted on the morning after an overnight fast. Measurement of Resting Metabolic Rate (RMR) using indirect calorimetry (COSMED Fitmate Pro), weight and body fat percentage (using Tanita Body Composition monitor) and height (using SECA stadiometer) were measured. Later, subjects were asked to consume the test food which contained either sugar or LCSs (stevia or aspartame). All test foods were identical except for sweetener content. Postprandial metabolic rate was measured at 3 intervals after test food was ingested; at 30 minutes, 60 minutes and 120 minutes. The protocol was repeated in the next 8 test session in at least 3 to 7 days interval each. Physical activity of the subjects was monitored; ideally subjects were restricted from doing any vigorous physical activities and to maintain normal daily routine especially within 24 hours before each test session.

Statistical analysis

Statistical analysis was done via IBM SPSS STATISTICS Version 22.0. Descriptive statistics presented for background data of participants (age, BMI, body fat percentage) and RMR measurements. To compare the changes in RMR between the different sweeteners, the paired t-test were used. Repeated measures ANOVA was used to measure the significance of RMR changes over time for all types of sweeteners.

Result:

A total of 28 participants completed their RMR assessment to determine their metabolic rate changes following consumption of the test samples. Table 3 shows the background of participants.

Table 3 Background of participants

	Age (year)	BMI (kg/m2)	Bodyfat (%)	RMR (kcal)
Mean ± SD	25.4 ± 4.04	23.0 ± 4.26	28.6 ± 5.54	1370 ± 287.7

Note: Result are represented by mean ± standard deviation (n=28).

Proximate analysis

For proximate analysis, the total carbohydrate, total protein, total fat, moisture, ash and energy content of the test samples was determined. Table 4 below shows the proximate analysis of chocolate muffin with different sweeteners; sugar, stevia and aspartame.

Table 4 Proximate analysis of chocolate muffin (sugar, stevia and aspartame)

Parameter	Total Carbohydrate, g/ 100g	Protein, g/ 100g	Total Fat, g/ 100g	Moisture, g/ 100g	Ash, g/ 100g	Energy, kcal/100g
Sugar	50.9	6.73	7.62	32.7	2.09	299
Stevia	31.3	8.22	9.99	47.8	2.69	248
Aspartame	31.0	7.90	10.3	48.3	2.54	248

Results showed varying contents in protein, fat, ash and moisture from all samples despite using the same recipe. The sugar containing test samples yielded the largest difference in total carbohydrate and energy compared to the stevia and aspartame samples (Table 4). High content of carbohydrate and energy at 50.9g/ 100g and 299 kcal/ 100g respectively compared to the stevia (total carbohydrate; 31.3g/ 100g and energy; 248 kcal/ 100g) and aspartame (total carbohydrate; 31g/ 100g and 248 kcal/ 100g). The result suggests an energy reduction due to sugar replacement with stevia and aspartame.

Metabolic changes

RMR assessment and analysis were done on 28 participants to determine the influence of type of sugar on the metabolic rate. Factors known to affect the metabolic rate were eliminated. RMR of subjects was recorded at 0 minutes pre consumption of the test foods and 30, 60 and 120 minutes post consumption. RMR content of the subjects are shown in kcal over time (Table 5).

Table 5 RMR assessment for chocolate muffins (Mean ± SD).

Type of LCS	RMR (kcal)			
	0′ Minute	30′ Minute	60′ Minute	120′ Minute
Sugar	1362±296[a]	1452±339[a]	1465±354[a]	1474±339[a]
Stevia	1368±314[a]	1498±361[a]	1446±305[a]	1449±298[a]
Aspartame	1364±342[a]	1472±396[a]	1526±283[a]	1428±292[a]

No significant difference according to Duncan multiple range test at $p < 0.05$.

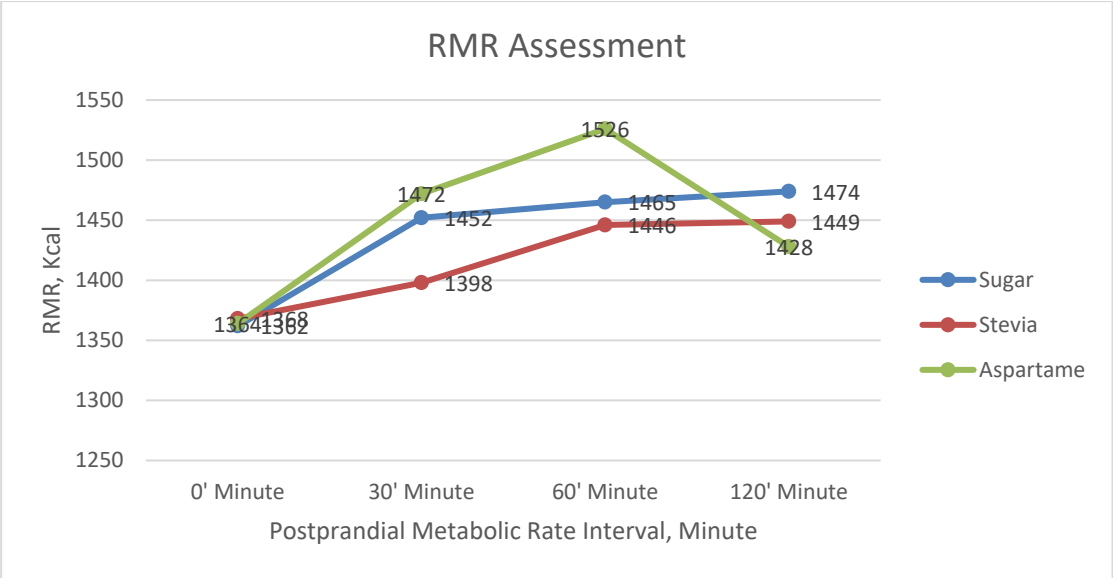

Figure 1 RMR assessment for chocolate muffins

Table 5 shows that at the 30′ minute postprandial, the RMR reading from stevia-based chocolate muffin consumption produced a higher amount of energy which is 1498 kcal, followed by aspartame and sugar, 1472 kcal and 1452 kcal respectively. At 60′ minute postprandial, the aspartame – based chocolate muffin readings are 1526 kcal followed by sugar and stevia which is 1465 kcal and 1446 kcal respectively. Finally, at 120′ minute postprandial, the RMR reading from the consumption of sugar – based muffin changed to 1474 kcal, followed by stevia and aspartame which is 1449 kcal and 1428 kcal. There were no significant changes among the three sweeteners; sugar, stevia and aspartame from chocolate muffin. Based on the hypothesis and the end results, stevia and aspartame's consumption do not reduce the metabolic rate that can subsequently reduce total energy expenditure. This study has revealed that metabolic rate fluctuates as the human body tend to change the parts of calories from other ingredients into energy even at lower energy intake and whether the foods contained non-nutritional sweetener or sugar.

Discussion:

Sylvetsky et al. (2016) commented in their review that LCS does not interfere with weight loss in the context of caloric restriction and may promote compliance, especially in individuals with a history of high sweetener intake. However, without concurrent, comprehensive, and sustained dietary modifications, it is unlikely that LCS will aid in weight loss when used in the general population. It means that LCS will not help in losing weight if there is no sustained dietary modification involved especially individuals with uncontrollable eating habits. They tend to eat more if the food and beverages contain LCS or is advertised as lower in calories. The energy quantity in foods or beverages will decrease by replacing the caloric to non-caloric sweeteners. Nevertheless, whether reducing energy density in this manner always translates into reduced energy intake, lower body weight, and improved metabolic health is much less certain. (Swithers, 2013). By replacing sugar with LCS, the reduction of energy density is far greater for liquid than it is for semi-solid foods or solid foods (chocolate muffin). This is because since sugar is the only main source of energy in liquid, LCS helps to reduce the energy density to 0 kcal/g compared to solid food (chocolate muffin). A study from Elnaga et al. (2016) evaluating the effect of stevia on weight management and several haematological and biochemical parameters of female rats, showed significant improvements in the depletion of final body weight, body weight gain (%) and feed efficiency ratio from the stevia sweetener group compared to the control group. Furthermore, compared to the control groups, the stevia sweetener group managed to lose weight, and reduce total cholesterol, triglycerides, and low-density lipoprotein concentration with the increment in the high-density lipoprotein. Rogers et al. (2016) reported that consuming Low Energy Sweetener, LES or known as LCS beverages does reduce energy intake and body weight, which means that there is no difference between the effect of water consumption and beverages sweetened with LES consumption.

Nonetheless, Bellisle & Drewnowski (2007) stated that replacing intense sweeteners only allows a meaningful reduction in the energy density of beverages where reduction is smaller for semi-liquid food products, and very small for solid foods. Livingstone et al. (2000) claimed that several factors

will yield negative outcomes that is absolute energy content or the differences in the macronutrient composition of the preload may not be sufficient to allow detection by physiological mechanisms. Therefore, the reduction of energy may be too small to make a change in metabolic rate after consumption. Stevia and aspartame, despite reducing the energy density of the foods still does not affect metabolic changes. Today, most consumers still do not receive enough information on LCS even though the industry has come a long way in providing various safe products to consumers. Although it is available, the pieces of information related to LCS provided are still inconsistent since it is favouring and depending on the motive of a certain body or industry providing the information.

Conclusion:

The amount of nutritional content among test foods that use sugar, stevia and aspartame are different from each other. Although the difference between sugar, stevia and aspartame is large, the result from metabolic assessment is still not enough to conclude that stevia and aspartame can reduce the subject's metabolic rate. Metabolic assessment is expected to show no difference in metabolic rate with ingestion of LCS compared to sugar. Hence, LCS consumption, although containing a negligible amount of calorie, does not significantly reduce the metabolic rate and this does not interfere with weight reduction through dietary restrictions.

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