Original Article

Salivary cariogenic bacteria counts are associated with obesity in student women at a Malaysian university

Wey-Zheng Yeo BSc, Sheng-Pei Lim BSc, Yee-How Say PhD

Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR) Kampar Campus, Perak, Malaysia

Background and Objectives: The counts of cariogenic bacteria lactobacilli and mutans streptococci have been studied and correlated with sugar intake. This study was to investigate the association between salivary lactobacilli and mutans streptococci counts with sweet food eating behavior and sweet sensitivity among 120 Malaysian women (101 ethnic Chinese, 19 ethnic Indians), while taking into account anthropometric and menstruation variables. **Methods and Study Design:** Demographics, anthropometric measurements and menstrual history were taken. Hedonic preference, intake frequency of a list of sweet foods, intensity perception and pleasantness ratings of sweet stimuli were assessed. Saliva was collected for lactobacilli and mutans streptococci culture. **Results:** We found that centrally obese subjects (high waist circumference and waist-hip ratio) had significantly higher salivary lactobacilli and mutans streptococci counts (all $p<0.05$), while overweight and high total body fat subjects had significantly higher salivary mutans streptococci counts ($p<0.001$). The sweetness intensity perception of chocolate malt drinks was significantly lower in women who were in their pre-menstrual (post-ovulation) phase. However, menstruation variables (menstrual phases, regularity and pre-menstrual syndromes) did not play a role in determining compulsive eating, sweets/chocolate craving and salivary lactobacilli and mutans streptococci counts. **Conclusions:** Taken together, salivary lactobacilli and mutans streptococci counts of the Malaysian women are associated with central obesity, but not sweet food eating behaviour, sweet sensitivity and menstruation variables. Salivary microbiome analysis could be useful as a potential diagnostic indicator of diseases such as obesity.

Key Words: lactobacilli, mutans streptococci, cariogenic bacteria, obesity, menstruation cycle

INTRODUCTION

People with a 'sweet tooth' are those who have a persistent desire to eat sweet products.[1] Malaysians are said to have one of the 'sweetest teeth' in the Asia Pacific region, with 43.3 kg/capita/year for the Food Supply Quantity for sugar and sweeteners in 2011 – way higher than China (7.4) and India (22.0), but still lower than Australia (46.5), Brunei (49.1) and New Zealand (54.8).[2] Increased caloric intake from sugar can cause energy imbalance and might have contributed to the high combined prevalence of overweight and obesity of 43.8% and 48.6% among men and women above 20 years in Malaysia, respectively.[3] However, it is controversial whether the increased preference of sweet foods (which may also lead to increased intake frequency) is correlated to body mass index (BMI). In our previous study, we showed that lean subjects significantly preferred more, took more frequently and craved more sweet foods compared to overweight subjects.[4] In relation to that, as a measurement of 'sweet tooth', we also assessed the intensity perception and pleasantness ratings of sweet stimuli among lean and overweight subjects, and found that there was no significant difference between them.[5] Indeed, most cross-sectional food surveys showed an inverse relationship between sugar intake and BMI[6-8] and under-reporting in the dietary survey has been attributed as the cause for it.[9,10] Thus, this calls for the need of an objective marker for sugar intake to be used complementarily in dietary surveys.

Lactobacilli (LB) and mutans streptococci (MS) are acid-producing and acid-resistant groups of bacteria which ferments carbohydrates (monosaccharide, disaccharides and starch), and sucrose fermentation by them into low pH lactic acid causes dental caries.[11] The counts of these cariogenic bacteria has been studied and correlated with sugar intake (reviewed in[12]). Barkeling et al (2001; 2002)[13,14] found that the salivary MS counts were higher in obese women in the absence of an association between reported habitual energy intake of sweet foods and BMI, probably due to under-reporting. In their studies also, they took into consideration the effect of menstruation variables such as menstrual phases, regularity and pre-

Corresponding Author: Dr Yee-How Say, Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR) Kampar Campus, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia.
Tel: +605-4688888 ext. 4505; Fax: +605-4661676
Email: sayyh@utar.edu.my; howsyh@gmail.com
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eating behaviors like compulsive eating and sweet foods craving.[13,14] Therefore, they proposed that salivary cariogenic bacteria counts could be a potential objective marker for sugar intake. However, the review by Vågstrand and Birkhed (2007)[12] concluded that there is limited to moderate scientific strength for the association between salivary cariogenic bacteria (LB and MS) and sugar intake, because of the presence of many confounding factors.

The role of salivary and gut microbiome in obesity has been shown in various studies.[15-19] There was increased abundance of the *Firmicutes* phylum bacteria (which LB and MS belong to) found in the saliva of obese subjects, compared to normal-weight subjects.[15,16] This indicates that the amount of LB and MS in the saliva could modulate obesity directly, independently and regardless of the amount of sugar intake.

Therefore, the aim of this study was to assess the hedonic preference and intake frequency of a list of 20 sweet foods, the intensity perception and pleasantness ratings of sweet stimuli and to enumerate the salivary counts of LB and MS in female students with different body compositions and menstruation variables. The association between salivary LB and MS counts with sweet foods eating behavior and sweet sensitivity while taking into account anthropometric and menstruation variables was then investigated.

METHODS

Subjects

A convenience sampling method was adopted for this study. Questionnaire and sample collection was carried out among students of Universiti Tunku Abdul Rahman (UTAR) Kampar campus from January to March 2013. The inclusion criteria for this study were female subjects who were not pregnant and without any medical history such as long-term medication, chronic medical treatment or had antibiotic treatment during the past 2 months. The demographic information collected from subjects included their age, gender, self-identified ethnicity. A total of 120 healthy and unrelated female subjects (101 ethnic Chinese, 19 ethnic Indians; mean age of 20.6±1.51 years) were recruited. This study has been approved by the UTAR Scientific and Ethical Review Committee (UTAR/SERC/2012/30May), all the subjects signed informed consent forms, and the study was conducted in accordance with the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

Questionnaire, sweet stimuli ratings, menstrual history and anthropometric measurements

A self-administered questionnaire on the preference, intake frequency and craving of a list of 20 types of commonly available sweet Malaysian foods and beverages was presented, as described in our previous study.[4] Pudding and jelly/jam on bread removed from original list as the ratings were too low across all subjects (all less than 2) and therefore would not add any value to statistical analysis. The sweet stimuli consisted of three suprathreshold concentrations of sucrose solutions of 3%, 7.5% and 18.75% w/v,[20] and two 3-in-1 chocolate malt drinks (Vico®) - less sugar (38 g sugars/100 g) and original (56 g sugars/100 g). The chocolate malt drink was prepared with boiling water, kept inside a thermos flask, and served lukewarm to the subjects. Subjects rated their intensity perception and pleasantness ratings using the general Labelled Magnitude Scale (gLMS)[21] and Labelled Affective Magnitude (LAM) scale,[22] respectively. Further details on the preparation of the stimuli and how sensory evaluation was conducted are as explained in our previous study.[5]

The subjects' menstrual history and complications were assessed based on the adaptations of questions from Barkeling et al (2001; 2002)[13,14] and Smith and Saunder (1969).[23] The age of first menstruation, current period of menstrual cycle (pre, menstruating or post), average regularity of menstrual cycle (28, >28 or <28 days) were assessed. General questions on sweet intake were also asked: "Do you consider that your intake of sweet foods is a problem for you and your weight"; "Do you eat more sweet foods during any part of the menstrual cycle".[13] If the subjects answered 'yes' to the latter question, they were required to state which period that they were more obsessed with sweet food eat more sweet food – before, during or after menstrual cycle.[14] Then, they were asked whether their menstrual cycle will influence their eating behavior. Subjects were also assessed on whether they feel like eating compulsively, develop craving for sweets and chocolates: during the time of their periods, when they are tense or depressed, and under normal circumstances.[23] Subsequent questions dealt with whether the subjects experienced tension/depression, pain and fluid retention during their menstrual periods.[23]

Anthropometric measurements and body compositions namely waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), weight, height, body mass index (BMI), total body fat percentage (TBF), visceral fat level (VFL), subcutaneous fat percentage (SF), skeletal muscle percentage (SM) and resting metabolism rate (RM) were measured as described in our previous study.[24] The cut-off points for overweight, overall adiposity (TBF) and central adiposity (WC and WHR) were ≥23 kg/m^2,[25] 30%,[26] 80 cm[25] and 0.85,[27] respectively.

Culture of cariogenic bacteria LB and MS

Subjects were given a 15-mL tube containing 5 mL of Reduced Transport Medium (RTF) buffer[28] as a transport medium for salivary bacteria collection. Self-collection of paraffin-stimulated whole saliva (of at least 3 mL) was performed by the subjects early in the morning (between 7 and 8 am) after they woke up and before toothbrushing and taking breakfast. Ten saliva samples were serially diluted in 10-fold dilutions with RTF buffer and 1 mL sample was plated on Mitis Salivarius Bacitracin (MSB) agar with 1% potassium tellurite solution[29] for MS and on de Man, Rogosa and Sharpe (MRS) agar[30] for LB for the pilot estimation of the optimal dilution for enumerable colony forming units (CFU). The MSB agar plates were incubated anaerobically in a chamber with GasPakTM, (BD, NJ, USA) at 37°C for 2 days and the MRS agar plates aerobically at 37°C for 3 days. The number of CFU of MS on MSB agar were counted and identified by their characteristic colony morphology,[31] while all CFU in MRS agar were regarded as LB. The enumerable dilu-

tions for MS and LB were 10-5 and 10-3, respectively. A free colony counter software (OpenCFU 3.8.11, available at http://www.softpedia.com/get/Science-CAD/OpenCFU.shtml) was used for the determination of CFU at the optimal enumerable dilutions for all the saliva samples.

Statistical analysis

The IBM SPSS Statistics software (IBM Inc., NY, USA) was used to analyze the data of the study. Descriptive statistics was used to calculate the frequencies and percentages. Categorical data were compared for significant association by Pearson's Chi-Square test. The normality of the continuous variables was checked using the Kolmogorov-Smirnov test and since they were not normally-distributed, non-parametric tests Mann-Whitney U (for 2 variables) or Kruskal-Wallis (for >2 variables) were used to compare means. All the means were presented as mean±standard error of the mean (SEM). Pearson's partial correlation test was used to correlate between two continuous variables, while controlling for ethnicity. The p-value of less than 0.05 was considered as statistically significant.

RESULTS

Relationship of salivary LB and MS counts with anthropometric classes and anthropometric measurements

Table 1 shows the means of salivary LB and MS counts for different ethnicities and anthropometric classes. Although Indian subjects had higher LB and MS counts than Chinese, the difference was not significant. Overweight and high TBF subjects had significantly higher MS counts but not LB counts, while subjects with high WC and WHR had significantly higher counts of both LB and MS. Table 2 shows that WC, WHR, TBF, SF and RM were significantly positively correlated with both salivary LB and MS counts, while weight, BMI and VFL were significantly positively correlated with salivary MS counts only. SM was not correlated with both salivary LB and MS counts. This indicates that with the increased obesity and body adiposity, salivary cariogenic bacteria like LB and MS might also increase.

Correlation of preference and intake frequency of sweet foods, and intensity perception and pleasantness ratings of sweet stimuli with salivary counts of LB and MS

In order to determine whether salivary counts of LB or MS could be predicted by an individual's 'sweet tooth', the preference and intake frequency of a list of common sweet foods and drinks in Malaysia, and the intensity perception and pleasantness ratings of sweet stimuli were assessed. Among the 20 foods in the list, only *apam balik* (a Malaysian pancake; normally with coarse sugar and peanut fillings) and *cendol* (a palm sugar-sweetened shaved ice dessert with condiments such as coconut milk, a worm-like jelly made from rice flour with green food coloring) had their preference and intake frequency showing significant correlation with either salivary counts of LB or MS, or both (Table 3). Referring to the overall means of the preference and intake frequency of *apam balik* and *cendol*, the subjects had a rather neutral to slightly positive preference rating, while they only had them around once a month or less often. Both preference and intake frequency of *apam balik* were significantly positively correlated with salivary LB counts, while only the latter was correlated with MS counts. Only preference of *cendol* was significantly positively correlated with salivary MS counts. The intensity perception and pleasantness ratings of all the sweet stimuli were not significantly

Table 1. Means of salivary counts of LB and MS for different ethnicities and anthropometric classes

Variable	n (%)	Mean±SEM (CFU/mL × 10^5)	
		LB	MS
Ethnicity			
Chinese	101 (84.2)	17.8±2.73	126±9.60
Indian	19 (15.8)	21.9±10.5	162±37.9
p		0.67	0.54
Overall obesity:			
Based on BMI			
Normal	69 (57.5)	16.1±3.55	92.6±11.0
Overweight	51 (42.5)	21.5±4.58	184±15.7
p		0.20	<0.01*
Based on TBF			
Normal	73 (60.8)	15.1±2.36	106±11.2
High	47 (39.2)	23.6±6.17	171±17.5
p		0.53	<0.01*
Central obesity:			
Based on WC			
Normal	73 (60.8)	13.0±2.32	108±11.4
High	47 (39.2)	26.9±6.08	168±17.5
p		<0.01*	<0.01*
Based on WHR			
Normal	88 (73.3)	14.1±2.67	119±11.4
High	32 (26.7)	30.2±7.32	166±20.2
p		<0.01*	0.01*

BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio TBF: total body fat; LB: lactobacilli; MS: mutans streptococci.
p-values by Mann-Whitney U test.
*p-value significant at <0.05.

Table 2. Correlation between salivary counts of LB and MS with anthropometric measurements

Anthropometric measurements	LB		MS	
	r	*p* value	*r*	*p* value
WC	0.25	0.01*	0.41	<0.01*
WHR	0.37	<0.01*	0.24	0.01*
Weight	0.17	0.07	0.37	<0.01*
BMI	0.15	0.11	0.43	<0.01*
TBF	0.21	0.02*	0.37	<0.01*
SF	0.20	0.03*	0.42	<0.01*
VFL	0.14	0.13	0.41	<0.01*
RM	0.17	0.07*	0.35	<0.01*
SM	-0.004	0.65	-0.15	0.11

WC: waist circumference; WHR: waist-to-hip ratio; BMI: body mass index; TBF: total body fat; SF: subcutaneous fat; VFL: visceral fat level; RM: resting metabolism rate; SM: skeletal muscle percentage; LB: lactobacilli; MS: mutans streptococci.
r and *p*-values by Pearson's partial correlation test, controlling for ethnicity.
*p-value significant at <0.05.

Table 3. Correlation of preference and intake frequency of two selected sweet foods with salivary counts of LB and MS

Sweet food	Mean±SEM of hedonic scale	Correlation with CFU/mL× 10^5			
		LB		MS	
		r	*p* value	*r*	*p* value
Apam balik					
Preference	4.15±0.15	0.20	0.03*	0.07	0.46
Frequency	2.02±0.06	0.30	<0.01*	0.22	0.02*
Cendol					
Preference	4.52±0.15	0.05	0.60	0.21	0.02*
Frequency	2.13±0.07	-0.09	0.33	-0.06	0.51

LB: lactobacilli; MS: mutans streptococci.
r and p-values by Pearson's partial correlation test, controlling for ethnicity.
*p-value significant at <0.05.

correlated with salivary counts of LB and MS (data not shown). This indicates that the subjects' preference, intake and sensory sensitivity of sweet foods did not influence their salivary LB and MS counts.

Effects of menstruation cycle variables on salivary LB, MS counts and sweet stimuli intensity perception and pleasantness ratings

The subjects reached their menarche at the mean age of 12.3±0.11. Majority of the subjects had the average length of menstrual cycle of >28 days. Fifty-nine percent (30 of 51) of the overweight women and 48% (33 of 69) of the normal-weight women considered their intake of sweet foods to be a contributory cause of their present weight (p=0.23). Thirty-seven percent (19 of 51) of the overweight subjects and 29% (20 of 69) of the normal-weight ones had extra large intake of sweet foods (p=0.34), where 26% of overweight women (5 of 19) and 30% (6 of 20) normal-weight women reported that they did that prior to menstruation. Except for the intake frequency of cake and the pleasantness rating of 3% sucrose solution, the preference and intake frequency of sweet foods, and intensity perception and pleasantness ratings of sweet stimuli were not significantly different between the normal-weight and overweight subjects (data not shown).

Table 4 shows that the salivary counts of LB and MS were not significantly different regardless of menstruation period and regularity, and the presence or absence of pre-menstrual tension, pain and fluid retention. The period of the menstrual cycle where the subjects were currently at when they performed the sweet stimuli sensory test also did not significantly affect the intensity perception and pleasantness ratings of the suprathreshold concentrations of sucrose solutions (Table 5), except for the intensity perception of regular and low-sugar chocolate malt drinks. A LSD post-hoc analysis on the log-transformed values of intensity perception of low-sugar chocolate malt drink further revealed that the ratings of those who performed the test during their post-menstrual period were significantly higher than those at their pre-menstrual period (p<0.001) or menstruating period (p=0.03). The influence of the different menstruation periods on the subjects' sweet food preference and intake frequency could not be assessed as they did not perform the daily record of food intake questionnaire. Nevertheless, Table 6 shows that those who ate compulsively and craved sweets and chocolate when depressed were not more likely to complain of pre-menstrual depression and fluid retention.

DISCUSSION

The summary of the findings of this study is presented in Figure 1. In the present study, more than half the overweight women considered that their intake of sweet foods was a contributory cause of their weight problems. However, they did not report a higher sweet food preference and intake frequency, and also were not less sensitive and did not report higher pleasantness rating towards sweet

Table 4. Means of salivary counts of LB and MS for various menstruation variables

Variable	n (%)	Mean±SEM (CFU/mL × 10^5)	
		LB	MS
Period			
Premenstrual	55 (45.8)	17.2±4.08	141±15.2
Menstruating	20 (16.7)	16.4±4.85	86.2±12.3
Post-menstrual	45 (37.5)	20.7±5.26	140±18.1
p		0.86	0.16
Regularity			
28	30 (25.0)	10.6±1.86	126±16.1
>28	67 (55.8)	21.2±4.52	131±15.7
<28	23 (19.2)	20.4±5.93	140±15.8
p		0.74	0.41
Tense/depressed during or before period			
Yes	81 (67.5)	19.5±3.53	144±13.6
No	39 (32.5)	16.1±4.70	104±11.7
p		0.86	0.12
More than average pain during or before period			
Yes	58 (48.3)	19.8±4.54	111±12.1
No	62 (51.7)	17.1±3.46	150±15.5
p		0.84	0.06
Premenstrual fluid retention			
Yes	30 (25.0)	25.4±7.22	145±23.7
No	90 (75.0)	16.1±2.88	127±10.9
p		0.09	0.80

LB: lactobacilli; MS: mutans streptococci
Means and *p*-values are by Kruskal- Wallis test or Mann-Whitney *U* test.

Table 5. Sweet stimuli intensity perception and pleasantness ratings during different periods of the menstrual cycle

Sweet stimulus	Mean±SEM (mm on gLMS/LAM scale)			*p*-value
	Pre-menstrual n=55	Menstruating n=20	Post-menstrual n=45	
3% sucrose solution				
Intensity	14.6±1.41	12.4±1.82	19.7±2.59	0.13
Pleasantness	45.1±2.75	45.9±2.78	50.0±2.31	0.72
7.5% sucrose solution				
Intensity	37.7±2.32	40.7±5.20	41.1±3.31	0.84
Pleasantness	45.5±2.40	49.5±2.38	43.8±2.74	0.49
18.75% sucrose solution				
Intensity	63.8±2.96	65.8±4.99	70.0±3.12	0.35
Pleasantness	35.3±2.97	39.8±4.07	30.8±3.26	0.16
Regular chocolate malt drink				
Intensity	34.6±2.84	35.6±3.93	47.0±3.70	0.04*
Pleasantness	55.0±2.69	63.1±3.57	59.4±2.56	0.27
Low-sugar chocolate malt drink				
Intensity	21.8±2.55	26.4±4.14	37.9±4.00	<0.01*
Pleasantness	56.2±2.42	60.4±3.26	59.8±2.44	0.48

Means and *p*-values by Kruskal-Wallis test.
*p-value significant at <0.05.

stimuli compared to that of normal-weight women. On the other hand, the overweight and high overall adiposity (high TBF) subjects had significantly higher salivary MS counts and also those who had high central adiposity (high WC and WHR) had significantly higher salivary LB and MS counts. Menstruation periods and PMS also did not seem to affect the general and sweet food eating behavior of the subjects directly, and hence the counts of salivary LB and MS indirectly. The high salivary LB and MS counts could reflect that those who had higher TBF, WC and WHR actually had higher intake of sweet foods in the real situation. Although we did not perform the habitual energy intake from sweet foods in the studies of Barkeling et al (2001; 2002),[13,14] we replicated their similar findings that there was lack of relationship between sweet food preference and intake frequency with anthropometric measurements indicative of obesity and adiposity. Barkeling et al (2001; 2002)[13,14] speculated that this could be due to under-reporting of the sweet food eating behavior, especially among the overweight and obese subjects. They reasoned out that sheer forgetfulness or social desirability in missing out/recording in small quantities of unhealthy foods[10] could be the reasons of under-reporting. Hence, they proposed that the salivary counts of LB and MS could be used as simple and cheap objective tests in validating the consumption of sweet foods. However, a review by Vågstrand and Birkhed (2007)[12] concluded that there is limited to moderate scientific

Table 6. Association of premenstrual depression and premenstrual fluid retention with compulsive eating and sweets/chocolate cravings during depression

During depression	Premenstrual depression				Premenstrual fluid retention			
	Yes n (%)	No n (%)	χ^2	*p*	Yes n (%)	No n (%)	χ^2	*p*
Eating compulsively			3.51	0.06			1.90	0.17
Yes	50 (61.7)	17 (43.6)			20 (66.7)	47 (52.2)		
No	31 (38.3)	22 (56.4)			10 (33.3)	43 (47.8)		
Crave for sweets			1.56	0.21			1.61	0.21
Yes	41 (50.6)	15 (28.5)			17 (56.7)	39 (43.3)		
No	40 (49.4)	24 (61.5)			13 (43.3)	51 (56.7)		
Crave for chocolate			0.52	0.47			0.04	0.83
Yes	41 (50.6)	17 (43.6)			14 (46.7)	44 (48.9)		
No	40 (49.4)	24 (56.4)			16 (53.3)	46 (51.1)		

χ^2 and *p*–values by Pearson's Chi-square test.

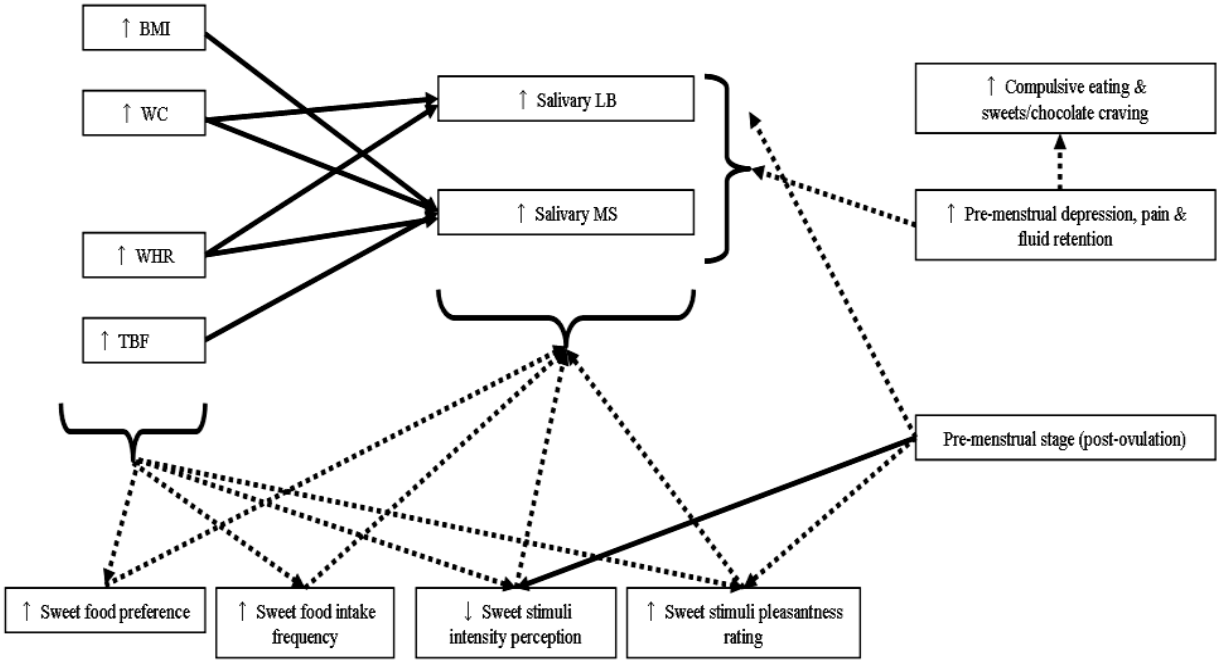

Figure 1. Summary of the findings of the study. Filled lines indicate positive association while dotted lines indicate no association. Increase in anthropometric measurements like body mass index (BMI), waist circumference (WC), waist-hip ratio (WHR), and total body fat percentage (TBF) are associated with increased counts of salivary cariogenic microbes (LB and MS). Women who were at their premenstrual stage (post-ovulation phase of the menstrual cycle) reported significantly lower intensity perception of chocolate malt drinks. Obesity and adiposity did not affect the sweet food preference and intake frequency, and the intensity perception and pleasantness ratings of sweet stimuli, which in turn also not associated with salivary LB and MS counts. Menstruation variables were not associated with eating behavior and salivary LB and MS counts.

strength for the association between salivary cariogenic bacteria (LB and MS) and sugar intake, because of the presence of many confounding factors. Therefore, the use of bacteria counts as a precise measurement or biomarker for sugar intake is still inconclusive and further investigation is warranted.

Another possible explanation for the lack of effect on the sweet food preference and intake frequency on salivary LB and MS counts is that the microbiota composition in the normal-weight and overweight subjects is different, independent of sugar intake. The salivary microbiota has been linked to obesity in Boston women,[19] and bacterial cellular abundance in oral subgingival biofilms was associated with obesity in Swedish adolescents.[15] Goodson et al (2009)[19] observed higher *Selenomonas noxia* (*Veillonellaceae*) presence in saliva of obese women. Furthermore, bacteria of the *Firmicutes* and *Actinobacteria* phyla and *Streptococcaceae*, *Gemellaceae* and *Enterococcaceae* families were more abundant in obese samples, whereas *Proteobacteria* and *Fusobacteria* phyla and *Fusobacteriaceae*, *Veillonellaceae*, *Prevotellaceae*, *Flavobacteriaceae* and *Lachnospiraceae* families dominated in normal-weight subjects.[16] As observed in previous studies,[15,16] we found increased counts of LB and MS (both of *Firmicutes* phylum, MS of *Streptococcaceae* family), in overweight subjects compared to those of normal-weight. Since considerable amounts of bacterial cells are swallowed daily with saliva,[32] the microbiota of saliva could therefore affect the gut microbiota composition. It is hypothesized that the higher abundance of *Firmicutes* in the saliva of overweight subjects could end up in the gut and promote absorption of monosaccharide, and

therefore may play a role in the development of obesity.[17,18]

In our study, we found that the intensity perception of the chocolate malt drinks (but not sucrose solutions) was highest among post-menstrual (or pre-ovulation) women, while their pleasantness ratings were the lowest (although not significantly). This indicates that they were more sensitive in sensing 'real-life' sweet stimuli during their post-menstrual period, rather than non-flavored sugar solutions. Nevertheless, Than et al (1993)[33] have indicated that sucrose detection threshold (or sensitivity) was increased by estrogen during pre-ovulation and decreased by progesterone during menstruation and post-ovulation, while the preference for sucrose apparently increased during luteal and menstrual phases, while decreased during follicular and ovulatory phases.[34] Animal experiments also showed the effect of estrogen on the increase of taste threshold for sucrose in rats.[35]

In this study, there were more women who suffered from depression but not pain and fluid retention. An association was found between craving and intake for food or sweets at specific times of menstrual periods or during the occurrence of PMS such as depression, pain and fluid retention.[13,14,23] However, we could not replicate this finding. Barkeling et al (2001; 2002)[13,14] proposed that PMS-related changes in mood and food intake is a culture-bound phenomenon as it is more well-known across the Western society, whereas women in tribal societies do not experience it.[36] Therefore, cultural differences in the influence of PMS on eating behavior might explain the discrepancies in the findings.

There were several limitations in this study. First, a comparatively small sample size, particularly with regards to Indian subjects and lack of Malay subjects, made the results difficult to generalize to the whole population of Malaysia. Next, the preference and intake frequency of sweet foods were obtained through questionnaire, thus, there is high probability of under-reporting, which may be due to inaccuracy in answering the questionnaire. In the future, saliva microbiota analysis of normal-weight and overweight/obese people could be performed by pyrosequencing, as culture-independent techniques are required to study the salivary microbial community since many of its members have not been cultivated.[37] The salivary microbiota could serve as a potential diagnostic indicator of not just only obesity,[15-16] but also diseases like oral cancer,[38] celiac disease[39] and dental caries.[40]

Conclusion

In conclusion, this study showed that the salivary counts of cariogenic microorganisms, namely LB and MS, but not the hedonic rating of preference and intake frequency of a list of sweet foods, were correlated with anthropometric measurements indicative of obesity and adiposity, namely BMI, WC, WHR and TBF. Menstruation variables such as menstrual phases, regularity and PMS also did not play a role in determining compulsive eating and sweets/chocolate craving and salivary LB and MS counts. It remains to be determined whether salivary LB and MS counts could be conclusively used as an objective indicator of sugar intake or 'sweet tooth' in the Malaysian population. Nevertheless, saliva microbiota analysis of normal-weight and overweight/obese people by culture-independent pyrosequencing could be useful as a potential diagnostic indicator of several diseases besides obesity.

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AUTHOR DISCLOSURES

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REFERENCES

1. Reed DR, McDaniel AH. The human sweet tooth. BMC Oral Health. 2006;6:S17. doi: 10.1186/1472-6831-6-S1-S17.
2. FAO (Food and Agriculture Organization of the United Nations). Food balance sheets. 2015/12/31 [cited 2016/06/27]; Available from: http://faostat3.fao.org/faostat-gateway/go/to/download/ FB/FBS/E.
3. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384:766-81. doi: 10.1016/S0140-6736(14)60460-8.
4. Sia BT, Low SY, Foong WC, Pramasivah M, Khor CZ, Say YH. Demographic differences of preference, intake frequency and craving hedonic ratings of sweet foods among Malaysian subjects in Kuala Lumpur. Mal J Med Health Sci. 2013;9:55-64.
5. Thai PK, Tan EC, Tan WL, Tey TH, Harpreet Kaur, Say YH. Perception and preference of sucrose, aspartame solutions and cola among multi-ethnic Malaysian subjects. Food Qual Prefer. 2011;22:281-9. doi: 10.1016/j.foodqual.2010.11.004.
6. Gibson SA. Are high-fat, high-sugar foods and diets conducive to obesity? Int J Food Sci Nutr. 1996;47:405-15. doi: 10.3109/09637489609006954.
7. Drewnowski A, Henderson SA, Shore AB, Fischler C, Preziosi P, Hercberg S. The fat-sucrose seesaw in relation to age and dietary variety of French adults. Obes Res. 1997;5:511-8. doi: 10.1002/j.1550-8528.1997.tb00571.x.
8. Macdiarmid JI, Vail A, Cade JE, Blundell JE. The sugar-fat relationship revisited: differences in consumption between men and women of varying BMI. Int J Obes Relat Metab Disord. 1998;22:1053-61. doi: 10.1038/sj.ijo.0800724.
9. Macdiarmid JI, Blundell JE. Dietary under-reporting: what people say about recording their food intake. Eur J Clin Nutr 1997;51:199-200.
10. Macdiarmid JI, Blundell JE. Assessing dietary intake: who, what and why of under-reporting. Nutr Res Rev. 1998;11:231-53. doi: 10.1079/NRR19980017.
11. Van Houte J. Role of micro-organisms in caries etiology. J Dent Res. 1994;73:672-81. doi: 10.1177/00220345940730031301.
12. Vågstrand KE, Birkhed D. Cariogenic bacteria as biomarkers for sugar intake. Nutr Rev. 2007;65:111-21. doi: 10.1111/j.1753-4887.2007.tb00288.x.
13. Barkeling B, Andersson I, Lindroos AK, Birkhed D, Rössner S. Intake of sweet foods and counts of cariogenic microorganisms in obese and normal-weight women. Eur J Clin Nutr. 2001;55:850-5.
14. Barkeling B, Linné Y, Lindroos AK, Birkhed D, Rooth P, Rössner S. Intake of sweet foods and counts of cariogenic microorganisms in relation to body mass index and

psychometric variables in women. Int J Obes Relat Metab Disord. 2002;26:1239-44. doi: 10.1038/sj.ijo.0802034.
15. Zeigler CC, Persson GR, Wondimu B, Marcus C, Sobko T, Modéer T. Microbiota in the oral subgingival biofilm is associated with obesity in adolescence. Obesity (Silver Spring). 2012;20:157-64. doi: 10.1038/oby.2011.305.
16. Piombino P, Genovese A, Esposito S, Moio L, Cutolo PP, Chambery A et al. Saliva from obese individuals suppresses the release of aroma compounds from wine. PLoS One. 2014;9:e85611. doi: 10.1371/journal.pone.0085611.
17. DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. Mayo Clin Proc. 2008;83:460-9. doi: 10.4065/83.4.460.
18. Tehrani AB, Nezami BG, Gewirtz A, Srinivasan S. Obesity and its associated disease: a role for microbiota? Neurogastroenterol Motil. 2012;24:305-11. doi: 10.1111/j.1365-2982.2012.01895.x.
19. Goodson JM, Groppo D, Halem S, Carpino E. Is obesity an oral bacterial disease? J Dent Res. 2009;88:519-23. doi: 10.1177/0022034509338353.
20. Keskitalo K, Knaapila A, Kallela M, Palotie A, Wessman M, Sammalisto S, Peltonen L, Tuorila H, Perola M. Sweet taste preferences are partly genetically determined: Identification of a trait locus on chromosome 16. Am J Clin Nutr. 2007;86:55-63.
21. Bartoshuk LM, Duffy VB, Green BG, Hoffman HJ, Ko CW, Lucchina LA, Marks LE, Snyder DJ, Weiffenbach JM. Valid across-group comparisons with labeled scales: the gLMS versus magnitude matching. Physiol Behav. 2004;82:109-14. doi: 10.1016/j.physbeh.2004.02.033.
22. Schutz HG, Cardello AV. A labeled affective magnitude (LAM) scale for assessing food liking/disliking. J Sens Stud. 2001;16:117-59. doi: 10.1111/j.1745-459X.2001.tb00293.x.
23. Smith SL, Sauder C. Food cravings, depression, and premenstrual problems. Psychosom Med. 1969;31:281-7.
24. Chan PM, Fan SH, Say YH. No association of Peptide Tyrosine-Tyrosine (PYY) gene variant R72T with obesity in the Kampar health clinic cohort, Malaysia. Mal J Nutr. 2011;17:201-12.
25. WHO/IOTF/IASO. The Asia-Pacific perspective: redefining obesity and its treatment. Hong Kong: World Health Organization, International Obesity Task Force, International Association for the Study of Obesity; 2000.
26. Omron. Omron body composition monitor with scale model HBF-362 Karadascan. Japan: Omron Healthcare Co. Ltd: 2008. pp. 42-5.
27. WHO. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008. Geneva: WHO Press; 2011.
28. Finlayson MH, Willey KF, Brede HD, Wilson AJ. A note on the use of reduced transport fluid (RTF) for isolation of Neisseria gonorrhoeae. S Afr Med J. 1974;48:1195-6.
29. Gold OG, Jordan HV, Van Houte J. A selective medium for Streptococcus mutans. Arch Oral Biol. 1973;18:1357-64. doi: 10.1016/0003-9969(73)90109-X.
30. de Man JD, Rogosa M, Sharpe ME. A medium for the cultivation of lactobacilli. J App Microbiol. 1960;23:130-5. doi: 10.1111/j.1365-2672.1960.tb00188.x.
31. Emilson CG. Prevalence of Streptococcus mutans with different colonial morphologies in human plaque and saliva. Scand J Dent Res 1983;91:26-32. doi: 10.1111/j.1600-0722.1983.tb00771.x.
32. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol. 2000 2005;38:135-87. doi: 10.1111/j.1600-0757.2005.00107.x.
33. Than TT, Delay ER, Maier ME. Sucrose threshold variation during the menstrual cycle. Physiol Behav. 1994;56:237-9. doi: 10.1016/0031-9384(94)90189-9.
34. Frye CA, Crystal S, Ward KD, Kanarek RB. Menstrual cycle and dietary restraint influence taste preferences in young women. Physiol Behav. 1994;55:561-7. doi: 10.1016/0031-9384(94)90117-1.
35. Curtis KS, Stratford JM, Contreras RJ. Estrogen increases the taste threshold for sucrose in rats. Physiol Behav. 2005;86:281-6. doi: 10.1016/j.physbeh.2005.08.002.
36. Johnson TM. Premenstrual syndrome as a western culture-specific disorder. Cult Med Psychiatry. 1987;11:337-56. doi: 10.1007/BF00048518.
37. Lazarevic V, Whiteson K, Gaïa N, Gizard Y, Hernandez D, Farinelli L, Osterås M, François P, Schrenzel J. Analysis of the salivary microbiome using culture-independent techniques. J Clin Bioinforma. 2012;2:4. doi: 10.1186/2043-9113-2-4.
38. Mager DL, Haffajee AD, Devlin PM, Norris CM, Posner MR, Goodson JM. The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. J Transl Med. 2005;3:27. doi: 10.1186/1479-5876-3-27.
39. Francavilla R, Ercolini D, Piccolo M, Vannini L, Siragusa S, De Filippis F et al. Salivary microbiota and metabolome associated with celiac disease. Appl Environ Microbiol. 2014;80:3416-25. doi: 10.1128/AEM.00362-14.
40. Yang F, Zeng X, Ning K, Liu KL, Lo CC, Wang W et al. Saliva microbiomes distinguish caries-active from healthy human populations. ISME J. 2012;6:1-10. doi: 10.1038/ismej.2011.71.