ORIGINAL ARTICLE

Correlation of cAMP Response Element Binding and Brain Derived Neurotrophic Factor Protein Levels in Adolescents with Adequacy Fruit Intake: A Cross Sectional Study

Siti Noor Syarma Mohd Sharif, Mohd Adzim Khalili Rohin, Norhayati Abd Hadi

School of Nutrition & Dietetics, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Gong Badak Campus, Hafsah Block, 21300 Kuala Nerus, Terengganu Darul Iman, Malaysia

ABSTRACT

Introduction: cAMP Response Element Binding (CREB) and Brain Derived Neurotrophic Factor (BDNF) protein levels tend to be a critical mediator for the beneficial effects of diet on neurogenesis and cognitive function. Increased fruit intake has been associated with improved cognitive function. However, in Malaysia, most adolescents still haven't met the recommended intake of fruits and far less is known on how their adequacy of fruit intake can be affected to their protein levels of CREB and BDNF. **Methods:** A cross sectional study was done on 352 students aged 14 and 16 years old involving 11 secondary schools in Kuala Terengganu, Marang and Hulu Terengganu Districts. A validated MyUM adolescent FFQ was used for fruit intake assessment. Blood sample to determine the level of CREB and BDNF proteins. **Results:** A total of 53.7% of adolescents consumed an adequate intake (≥ 2 servings/day) of fruits. Serum level BDNF was significantly ($p<0.001$) higher in the adolescent with adequate fruit intake (389.05±204.96 pg/mL) compared with inadequate (< 2 servings/day) group (118.60 ± 49.04 pg/mL) and was correlated positively with fruit intake ($r=0.62$, $p<0.001$). The median of CREB protein level was significantly ($p<0.021$) higher among inadequate intake of fruits (19.85±23.60 ng/mL) relative to adequate group (14.71± 17.9 ng/mL) and was negatively correlated with fruit intake (rs (8) =-0.07, $p<0.001$). Apple, papaya, watermelon, orange, pear, guava, mango, banana and durian were significant factors for BDNF protein level. **Conclusion:** High serum protein level of BDNF in the adolescent with adequate intake of fruit could have a role in controlling neuronal survival, and synaptic function in the central nervous system.

Keywords: Correlation, CREB, BDNF, Fruit Intake, Adolescents

Corresponding Author:
Mohd Adzim Khalili Rohin, PhD
Email: mohdadzim@unisza.edu.my
Tel: +609-6688522

INTRODUCTION

Fruits are known as a key factor in a healthy diet due to their healthy nutrients and biologically active compounds, including vitamins, minerals, fiber and phytochemicals (1). A minimum daily intake of 400g of fruits and vegetables is recommended in reducing the risk of cardiovascular disease, cancer and cognitive impairment, as well as to prevent micronutrient deficiencies (2). Malaysian Dietary Guidelines 2010 (MDG 2010) have recommended at least two servings of fruits among Malaysians over two years of age (3).

The main regulator of memory and learning processes is hippocampus (4). Positive impact of fruit intake has been reported in most recent reviews in animal and human studies (5), particularly anthocyanin and flavonol intake in enhancing both hippocampal cAMP Responsive Element-Binding (CREB) protein activity and Brain Derived Neurotrophic Factor (BDNF) protein levels (6-8). Fruits and vegetables are involved in neuronal plasticity through activation of CREB proteins, protein kinases and receptors in synapse (9–11), modulating the pathway of signaling and transcription factors. Phosphorylation of these kinases regulates synaptic efficiency (12,13). Whereas BDNF is a neurotrophin associated with the maintenance, survival, development, and differentiation of neurons (14). In fact, BDNF is so essential for cognitive processes in the brain that deletion of the BDNF gene weakens memory retention and impedes long-term potentiation (LTP) (15). Therefore, both CREB and BDNF protein levels are recognized to be required during memory acquisition and consolidation (16). Moreover, Huston et al. (17) pointed out that these two levels of molecular proteins can play a key role in the positive impacts of diet on cognitive function and neurogenesis (17). Other authors have also reported interventional studies that show their improvement through brain blood flow and pathways related to BDNF or CREB proteins. In this context, it is necessary to consume fruits and vegetables sufficiently to maintain normal cognitive functions (4).

However, fruits consumption among children and adolescents are still low in globally. A total of 60% children and adolescents did not achieved the serving sizes of fruit in the United States, even though the increasing trend was significant (12%) in total fruit intake from 2003 to 2010 (18). A similar situation in Asian countries was also reported that 76.3% for the adolescent population did not meet the recommended intake of fruits and/or vegetables per day (19). In Malaysia, majority of the adolescents (68.5%) still did not meet the recommended intake of fruits (20). Decreasing trend had been found in fruit consumption among adolescents in Malaysia, from 48.4% in 2012 to 31.5% in the year 2017 (20).

Adolescents have significant neuronal plasticity and show high rates of cell replacement (4). Given that low intake of fruit during adolescence may potentially associated with greater nutritional risk (21), long-lasting effects on memory and learning (17), and cognitive dysfunction during aging (22), increasing its consumption among adolescent is therefore a public health priority. In addition, most previous studies highlighted the beneficial effect of fruit polyphenols on memory and cognitive such as grapes, berries, strawberry, pomegranate and citrus fruits. To our knowledge, there is limited evidence for a positive effect on brain cells and neuronal function with other tropical fruits, and little is known about how the intake of these fruits affects CREB and BDNF protein levels in Malaysian adolescents. Therefore, current study aims to determine the prevalence of fruit consumption and estimate the difference in the serum level of CREB and BDNF in Terengganu adolescent with adequate and inadequate fruit intake.

MATERIALS AND METHODS

Study design and population

This was a cross sectional study conducted at 11 selected secondary schools in Kuala Terengganu, Marang and Hulu Terengganu Districts, from Jun 2018 until May 2019. The inclusion criteria were healthy students age 14 and 16 years old, having parental consent and the student was willing to take part. Students were excluded if they were anemic or had physical or medical conditions that influence normal dietary intake. Eligible students who fit the criteria received an information sheet, and they provided their sociodemographic profiles (gender, age, parent's occupation, household income and parent's education level) and parent's consent before the data collection.

The sample size for this study was calculated using single proportion formula using Epi tools program. Based on 31.5% of adolescents in Malaysia had adequate intake of fruit (20), 95% confidence level and 5% probability of missing a true difference, a minimum 332 respondents were required. After accounting for 20% attrition rate, sample size calculated was 415. In this study, a multistage random sampling method was used. Out of 415 information sheet distributed to school, 84.8% subjects returned completed information (n = 352). The total number of respondents was still acceptable as the minimum requirement from the sample calculation without dropout rate was 332. The ethic for this research was being obtained from the UniSZA Human Research Ethics Committee (UHREC) with a study protocol code (UniSZA/UHREC/2018/78). The study was also being approved by the Ministry of Educational Malaysia with register number, KPM.600-3/2/3-eras(1316).

Data Collection

School visit had been conducted for the fasting blood collection early in the morning. A total of 3 mL blood samples were collected from the respondent's antecubital veins in a plain tube by trained and experienced phlebotomists from Health Student Centre, Universiti Sultan Zainal Abidin. Protocol of handling the blood collection was based on the WHO Guidelines on Drawing Blood for Best Practices in Phlebotomy (23). After whole blood was collected, serum was separated by centrifugation at 1800xg for 10 minutes at 4°C in the laboratory, aliquoted, and stored at -80°C until further analysis. Serum blood samples were used for BDNF and CREB protein levels test. Then, the respondents were face to face interviewed using a validated MyUM Adolescent Food Frequency Questionnaire (FFQ) (24). Respondents were asked about the frequency of taking fruit diet within the past 12 months. A total of 15 common fruit lists were questioned and they would need to recall the fruit intake according to the frequency selection list. These fruit items were selected based on the comparative validity study of the MyUM Adolescent FFQ which was developed specifically for the Malaysian adolescent population (24). Frequency of fruit consumption were listed in nine optional columns (none per month; one to three times per month; one time a week; two to four times per week; five to six times per week; one time a day; two to three times per day; four to five times per day; and six and more times per day). For each fruit item listed, respondents must choose only one column, and placed one checkmark in that column that best describes how often they ate a given fruit item. Fruit model and a pictorial illustration were provided to help them in estimating the serving size of each portion based on the Malaysian Atlas of Food Exchanges and Portion Sizes (25).

Measurement

Determination of Cyclic AMP-Response Element Binding (CREB) Protein level

CREB levels were assessed using the Phospho-CREB (Ser133) sandwich ELISA kit (Cell Signaling Technology, USA). Materials were prepared following the standard and specific method of sampling. 100 µL of serum was added to the well, covered with plate sealer, and left overnight at 4°C or for two hours at room temperature. The wells were then being washed with 200 µL of 1X Wash Buffer (three times) and 100 µL of reconstituted Detection Antibody was added to each well, sealed with tape and re-incubated for one hours at 37°C. The washing procedure was repeated and 100 µL of TMB Substrate was added to each well, sealed with tape and re-incubated at 37°C for ten minutes. Each well were added with 100 µL Stop Solution to stop the reaction and the measurement for the absorbance at 450 nm wavelength using a flourometer micro-plate reader (TECAN, INFINITE M200) was run.

Determination of Brain-Derived Neurotropic Factor (BDNF) level

BDNF levels were assessed using the BDNF ELISA Assay (Abacam, UK). All equipment, standard and samples were prepared according to the environment specific method. 100 µL of each standard and serum samples were added to 96 well plates which were coated with antibody specific for human BDNF. The wells were covered and incubated at room temperature for 2.5 hours or overnight at 4°C with gentle shaking. Subsequently, the wells were washed with 100 µL of 1x Wash Buffer (3x). Then, 100 µL of 1x biotinylated anti-human BDNF detector antibody were added to each well and were incubated for an additional 1 hour. After wash with 1x Wash Buffer (3x), 100 µL of 1X HRP-Streptavidin solution was added to each well and re-incubated for 45 minutes at room temperature with gentle shaking. Then, solution was discarded and washed with 1x Wash Buffer and 100 µL of TMB one-step substrate reagent was added and re-incubated for 30 minutes at room temperature in the dark with gentle shaking. The reactions stopped by added 50 µL Stop Solution to each well and absorbance was measured at 450 nm wavelength using a flourometer micro-plate reader (TECAN, INFINITE M200).

Food Frequency Questionnaire Assessment

To assess the adequacy intake of the fruit groups, the original portion size of each fruit item was translated to the recommended size according to the Malaysian Dietary Guidelines (3). The amount consumed was calculated by multiplying the standardized serving size of the food group with the conversion factor of the reported frequency. The total intake (servings/day) of fruit was determined and compared with the amount of servings recommended in the reference (26). Adolescents were categorized as meeting the recommendations if their intake achieved two servings of fruit (26).

Statistical Analysis

Statistical Analysis was performed using the SPSS version 23.0. Descriptive data were expressed in frequency (percentage). Numerical data were presented as mean (SD) or median (IQR) based on their normality distribution. Independent t-test was used to compare difference in the serum level of CREB and BDNF in Terengganu adolescent with adequate and inadequate fruit intake. Pearson's and Spearman's rank correlation coefficient and simple linear regression were used to measure relationship between fruit intake with CREB and BDNF protein levels. The significance level for all statistical tests was set at $p<0.05$.

RESULTS

Prevalence of Fruit Intake Among Adolescents

From a total of 352 respondents, age categories were almost similarly distributed among 14 years old (n= 175, 49.7%) and 16 years old (n= 177, 50.3%) (Table I). Female respondents were slightly higher (n=195, 55.4%) than male (n=157, 44.6%). Majority of their parental education completed their secondary education from both fathers (n=331, 65.6%) and their mothers (n=244, 69.3%). About 191 of their fathers working as a self-employed (54.3%) while majority their mothers were housewife (n=258, 73.3%). Half of them were from RM1000 – RM3000 family household income (n=187, 53.1%).

Current study showed that about 189 adolescents met the daily recommended intake of 2 servings of fruits (53.7%) (Figure 1). The top five most commonly

Table I: Socio-demographic characteristic of respondents (n=352)

Variable	n	%
Gender		
Male	157	44.6
Female	195	55.4
Age (years)		
14	175	49.7
16	177	50.3
Parents Education Level		
Father		
Primary Education	42	11.9
Secondary Education	231	65.6
College/University	79	22.4
Mother		
Primary Education	44	12.5
Secondary Education	244	69.3
College/University	64	18.2
Parents Occupation		
Father		
Civil Servant	82	23.3
Private Sector	79	22.4
Self-Employed	191	54.3
Mother		
Civil Servant	65	18.5
Private Sector	29	8.2
Housewife	258	73.3
Household Income (RM)		
< RM1000	77	21.9
RM1000 – RM3000	187	53.1
RM3000 – RM8000	58	16.5
>RM8000	30	8.5

RM-Ringgit Malaysia

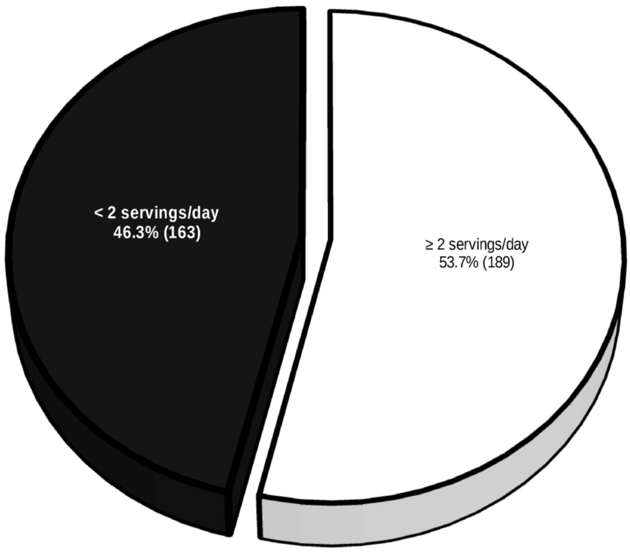

Figure 1: Prevalence of fruit intake among respondents. The prevalence of respondents who had adequate intake of fruit is higher compared to inadequate intake. Data are given as percentage (n = 352).

consumed fruits among adolescents in Terengganu were banana (22%), jackfruit (18%), watermelon (10%), rambutan (10%) and mango (8%) (Figure 2). According to the findings in Table II, there was higher percentage of female adolescents (n=112, 57%) who met the MDG 2010 recommendation compared to male (n=77, 49%). About 101 adolescents aged 16 years old tended to consume adequate fruits (57%) as compared with 88 adolescents aged 14 years old (50%). There was a higher percentage of adolescents from their parental primary educational level (father; n=22, 52%, mother; n=26, 59%) who consumed inadequate fruit intake. Overall, the percentages of adolescents from different parental occupation background who met the MDG 2010 recommendation of at least 2 servings of fruits intake per day were almost the same. By household income, higher percentage of adolescents from family household income >RM8000.00 (n=19, 63%) ate adequate fruits compared with others. While majority adolescents from rural area (n=113, 55%) tended to consume fruit less than recommended.

Table II: Prevalence of fruit intake among respondents by sociodemographic characteristics (n=352)

	< 2 servings/day		≥ 2 servings/day	
	n	% (95% CI)	n	% (95% CI)
Overall	163	46 (0.41, 0.52)	189	54 (0.48, 0.59)
Gender				
Male	80	51 (0.43, 0.59)	77	49 (0.41, 0.57)
Female	83	43 (0.36, 0.50)	112	57 (0.50, 0.64)
Age (years)				
14	87	50 (0.42, 0.57)	88	50 (0.43, 0.58)
16	76	43 (0.36, 0.50)	101	57 (0.50, 0.64)
Parents Education Level				
Father				
Primary Education	22	52 (0.37, 0.68)	20	48 (0.32, 0.63)
Secondary Education	107	46 (0.40, 0.53)	124	54 (0.47, 0.60)
College/University	34	43 (0.32, 0.54)	45	57 (0.46, 0.68)
Mother				
Primary Education	26	59 (0.44, 0.74)	18	41 (0.26, 0.56)
Secondary Education	106	43 (0.37, 0.50)	138	57 (0.50, 0.63)
College/University	31	48 (0.36, 0.61)	33	52 (0.39, 0.64)
Parents Occupation				
Father				
Civil Servant	37	45 (0.34, 0.56)	45	55 (0.44, 0.66)
Private Sector	41	52 (0.41, 0.63)	38	48 (0.37, 0.59)
Self-Employed	85	45 (0.37, 0.52)	106	55 (0.48, 0.63)
Mother				
Civil Servant	30	46 (0.34, 0.59)	35	54 (0.41, 0.66)
Private Sector	14	48 (0.29, 0.68)	15	52 (0.32, 0.71)
Housewife	119	46 (0.40, 0.52)	138	54 (0.48, 0.60)
Household Income (RM)				
< RM1000	35	45 (0.34, 0.57)	42	55 (0.43, 0.66)
RM1000 – RM3000	92	49 (0.42, 0.56)	95	51 (0.44, 0.58)
RM3000 – RM8000	25	43 (0.30, 0.56)	33	57 (0.44, 0.70)
>RM8000	11	37 (0.18, 0.55)	19	63 (0.45, 0.82)
Location of Residence				
Urban	50	34 (0.27, 0.42)	95	66 (0.58, 0.73)
Rural	113	55 (0.48, 0.61)	94	45 (0.39, 0.52)

RM-Ringgit Malaysia

Correlation of Fruit Intake with CREB and BDNF Protein Levels

There was significance difference in mean CREB and

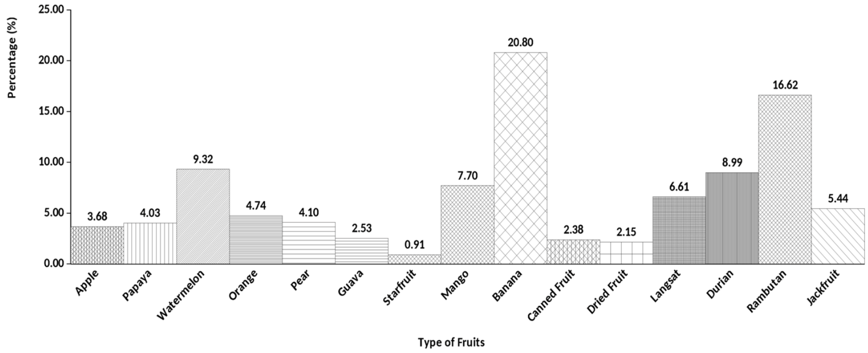

Figure 2: Distribution of fruits consumed among respondents. The bar chart of fruit intake analysis shows representative data of the type of fruits most consumed among respondents. Banana is the most consumed following by rambutan, watermelon, durian and mango. Data are given as percentage (n = 352)

BDNF protein level between adolescents who consume less than two servings of fruit daily and adolescents with adequate intake of fruit. According to the findings in Table III and Table IV, serum level BDNF was significantly higher in the adolescents with adequate fruit intake (389.05±204.96 pg/mL) and was correlated positively with fruit intake (r=0.62, p<0.001). The mean of CREB protein level was significantly (p<0.001) higher among adolescents with an inadequate intake of fruits (19.85±23.60 ng/mL) and was negatively correlated with fruit intake (rs (8) =-0.07, p<0.001). While in Table V, fruit intake and several type of fruits (apple, papaya, watermelon, orange, pear, guava, mango, banana and durian) were significant factors for BDNF protein level. There was no significant association found in CREB protein level with fruit intake and type of fruits.

Table III: Comparison of mean CREB and BDNF protein level between two groups of adequacy fruit intake

	< 2 servings/ day	≥ 2 servings/ day	Mean Difference (95% CI)	t-statisic[a] (df)	*P*-value
	Mean (SD)	Mean (SD)			
CREB (ng/ml)	19.85 (23.60)	14.71 (17.9)	-5.14 (-9.50, -0.79)	-2.32 (350)	0.021
BDNF (pg/ml)	118.60 (49.04)	389.05 (204.96)	270.45 (238.10, 302.80)	16.44 (350)	<0.001

[a]Independent t-test was applied

Table IV. Correlation between fruit intake with CREB and BDNF protein levels

	r	P-value
CREB (ng/ml)	-0.07	<0.001*
BDNF (pg/ml)	0.62	<0.001**

*Spearman's rank correlation coefficient

**Pearson's correlation coefficient

Table V: Associated factors of BDNF protein level with serving and type of fruit intake among respondents (n=352) by simple linear regression

Variables	Simple linear regression		
	b	(95% CI)	p-value
Fruit (Serving/day)	64.68	(56.01, 73.34)	<0.001
Apple (g)	1.50	(0.41, 2.57)	0.007
Papaya (g)	2.48	(1.69, 3.27)	<0.001
Watermelon (g)	1.05	(0.63, 1.47)	<0.001
Orange (g)	1.47	(0.89, 2.04)	<0.001
Pear (g)	0.58	(0.04, 1.12)	0.035
Guava (g)	1.93	(0.51, 3.35)	0.008
Mango (g)	1.21	(0.75, 1.67)	<0.001
Banana (g)	0.50	(0.33, 0.67)	<0.001
Durian (g)	0.49	(0.18, 0.79)	0.002

DISCUSSION

In this study, prevalence of fruit intake among adolescents in Terengganu was observed. Prevalence of adequate intake of fruits in current study (53.7%) was quite high compared to national level in NHMS 2017 (31.5%). However, the finding was comparable with NHMS 2012, where Terengganu was the highest intake of adequate fruits (56.5%) among other states (27). Similar findings of fruits intake among adolescents in Terengganu (20) regarding gender and age where female and older age group were found slightly higher consumed adequate fruit in current study. In contrast, according to national study, the percentages of adolescents from schools between rural areas and urban areas as well as between boys and girls who met the MDG 2010 recommendation of at least 2 servings of fruits intake per day were almost the same (20).

In the present study, 55% of adolescents in rural areas do not meet the recommendation for fruit consumption. This can be caused by a number of factors. According to a qualitative insight from the perspective of rural and urban adolescents in Kedah, Terengganu and Selangor, the main factors consistently cited as their major influence on the food choice were availability, accessibility, self-awareness and cost of food. These factors are important when making decisions about food choices (28). Generally, some low-income families living in rural areas had little access to shops or supermarkets but were surrounded by fruit trees and gardens, which can explain this consumption by the presence of fruits (29). However, adolescents' eating habits have been reported to have a significant impact on school eating environments (28). Access to food within or outside the school is an important determinant of food selection and food quality for adolescents as they spend more time at school (30). In addition, previous study reported that majority of 55% Malaysian adolescents in rural area revealed that fruit is not sold in school (28). This is supported by Terry-McElrath et al. (31) that the frequency of the supply of fruits and vegetables at school is positively and significantly related to the daily intake of adolescents. Furthermore, parents also play an important role in shaping adolescents' eating habits that can have a lasting effect on their health (28). Students whose parents belonged to higher educational and professional categories reported that they eat significantly more fruits and vegetables (32). Lower fruit intake in rural area might also influenced by these factors as current findings showed that percentages of family with household income less than RM1000 (26.6%) and primary educational level among parents (33.8%) were slightly higher compared to urban area which is 21.9% and 11.0% respectively. This group may not have enough knowledge about the standard portion size as recommended in MDG 2010. According to previous study indicated that the rural populations and people with lower education level cannot access this guideline easily (33). Therefore, they may consume fruit but not in proper adequate intake suggested. Similarly, the most commonly consumed fruits among adolescents in current and previous study (20, 34) were banana, watermelon and mango. This may be due to availability and affordability factors as well as their personal preference regarding its acceptability factors such as taste, color, odour and appearance.

Current study showed that, serum level BDNF was significantly higher in the adolescents with adequate fruit intake and was positively correlated with fruit intake. This was also proven by Chan et al., (5) indicated that significant correlation between serum BDNF level with higher frequency of fruit intake in his human cross sectional study. The relatively higher level of BDNF protein with adequate intake of fruit compared to inadequate serving of fruit consumed is believed to be due to its bioactive compound, vitamins or other substances in fruit that might affect circulating BDNF. Moreover, similar finding in almost interventional studies reported that there was positive relationship between BDNF levels, fruit intake and cognitive performance (4, 7, 8, 35, 36). In a small cross-sectional study of children conducted by Riggs et al., (37), there was no association between fruit consumption and executive functioning. However, two large cross-sectional studies have shown a positive association between fruit and executive functioning (38, 39).

Comparatively, in contrast with other studies, CREB protein level was significantly higher among adolescents with an inadequate intake of fruits and was negatively correlated with fruit intake. Most previous interventional studies have highlighted the beneficial effects of flavonoid fruits on enhancing regional working memory with coexisting increases in CREB activity and BDNF levels at the hippocampus (6-9, 11, 16, 40). CREB and BDNF are located downstream in the extracellular signal-regulated kinase pathway (41). The phosphorylation of CREB at Ser133 promotes gene transcription, which regulates the growth or regeneration of neurons and synaptic plasticity (42). CREB binding sequences have been identified in various genes, including BDNF encoding. In fact, there is a bidirectional regulation between BDNF and CREB that BDNF can be up regulated by CREB (42). In general, the activation of PKA, CaMKs and the Ras-ERK pathway can all lead to the phosphorylation of CREB. However, inverse relationship of protein level between CREB and BDNF in current finding may occurred due to previous studies which have reported that increase in BDNF expression induced by neuronal activity to be more dependent on other transcriptional activity (43).

The significant negative correlation result between CREB and fruit intake may also due to low-flavonoid fruit intake among respondents. As reported in previous interventional study indicated that there were no changes of low-flavonoid (<5 mg/100 g) fruit intake on that serum protein levels compared to significant impact of high flavonoid (>15 mg/100 g) fruit intake over an 18 – week period (8). This observation, broadly agrees with previous studies suggesting that flavonoid interventions seem to interact primarily with ERK and Akt pathways, leading to modulation of the transcription factor CREB (6-9, 11, 16, 40) as well as up-regulation of CREB gene expression (42). High flavonoid fruits have been documented in different berries and grapefruits varieties where the total flavonoid content are in the range of 6.32 mg/100 g to 529.95 mg/100 g (44). However, in current study, the concentrations of flavonoids encountered in papaya, watermelon, pear, guava, mango and banana were reported previously less than 5 mg/100 g (44). Flavonoid rich fruits are only found in apple, orange and durian. Three main classes of polyphenols in apple fruit, namely anthocyanidin, flavanol and flavonol contain up to 60.99 mg/100 g of total flavonoid (44). Total flavonoid content of different durian varieties is in the range of 1.90 mg/100 g to 93.90 mg/100 g (45). While a total of 43.04 mg/100 g flavonoid content in orange belongs to three major classes which are flavanones, flavones and flavonol (44). Nevertheless, to date, no published reports have been published on the relevance of fruit intake and its association with the CREB protein factor among healthy adolescents. It is unknown if antioxidants in fruits, vitamins or other substances can affect circulating CREB. It can be hypothesized that sufficient or excessive fruit intake can reduce CREB protein levels more than with a low fruit intake. Since this is a cross-sectional study design, future studies of the mechanisms underlying the relationship between these two areas are needed.

Significant associated factors of apple, papaya, watermelon, orange, pear, guava, mango, banana and durian with BDNF protein level indicated positive insight in promoting local tropical fruits for more enlightenment among Malaysian adolescents on the benefit of fruit intake to their cognitive function. Tropical fruits are important sources of nutrients such as vitamin A, vitamin E, vitamin B6, potassium, magnesium, iron, thiamine, niacin, as well as dietary fiber (46). Moreover, they are also biologically active compounds and are regarded as potential nutraceutical foods, partly due to antioxidant activity leading to polyphenol and / or ascorbic acid (47). For example, guava, mango, papaya and starfruit are good sources of vitamin A and high levels of vitamin C and carotenes compared to orange or lemon (46). Bananas are rich in potassium, a crucial element to maintain normal blood pressure and heart function. While, sterol content in bananas supports the cardiovascular system in humans by blocking the absorption of cholesterol, helping to keep blood cholesterol levels under control (48). Though watermelon has very low calories being all water. Furthermore, watermelon also contains essential minerals such as potassium and magnesium (49). In fact, the substances derived from these tropical fruits have been proven to preserve brain's optimal function especially in enhancing the BDNF protein level (17, 50). This current finding has enabled Malaysian adolescents to observe cognitive function related to fruit intake and quantitative assessment of fruit intake, whose data are still limited to this date.

CONCLUSION

Adequate intake of fruits was found to contribute significantly to BDNF and CREB protein level among adolescents in Terengganu. These results suggest that high intake of fruit may improve serum protein levels of BDNF, which may have a role in regulating neuronal survival and synaptic function in the central nervous system in adolescents. Local tropical fruits in Malaysia such as apple, papaya, watermelon, orange, pear, guava, mango, banana and durian were associated significantly in BDNF protein. Negative correlation was found between fruit intake and CREB protein level. These findings provide insight into what fruits Terengganu adolescents are consuming and the difference status in the serum protein level of CREB and BDNF among them with adequate and inadequate fruit intake.

Despite higher percentage of adolescents consumed adequate intake of fruits in current findings, lower prevalence in rural area should be concerned more for targeting the strategies to enhance their consumption in fruit intake. These data provide health professionals and policy makers with important background information that develops appropriate and effective strategies for promoting fruit intake as part of Malaysian adolescents' healthy eating habits. Future studies are needed for interventional studies regarding dietary fruit intake, cognitive function as well as effects on behavior in understanding the cellular and molecular mechanism of CREB-BDNF related pathways.

ACKNOWLEDGEMENTS

The researchers would like to express our gratitude to the Ministry of Higher Education Malaysia, Ministry of Education Malaysia and Universiti Sultan Zainal Abidin (UniSZA) for the financial aid (UniSZA/2018/DPU/14) and the participants for their cooperation and contribution towards this study. The authors would also like to acknowledge all staffs from Faculty of Health Sciences and Faculty of Medicine, UniSZA.

REFERENCES

1. Liu, R.H. Health-promoting components of fruits and vegetables in the diet. Adv. Nutr. 2013;4:384S-392S.
2. Rodriguez-Casado, A. The health potential of fruits and vegetables phytochemicals: notable examples. Crit. Rev. Food Sci. Nutr. 2016;56:1097–1107.
3. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia. Malaysian Dietary Guidelines. Putrajaya, Malaysia: Ministry of Health Malaysia;2010.
4. Carillo JA, Zafrilla MP, Marhuenda J. Cognitive function and consumption of fruit and vegetable polyphenols in a young population: is there a relationship? Foods.2019;8:507-524.
5. Chan KL, Tong KY, Yip SP. Relationship of serum brain-derived neurotrophic factor (BDNF) and health-related lifestyle in healthy human subjects. Neurosci Lett. 2008;447(2–3):124–8.
6. Williams CM, Abd M, Mohsen E, Vauzour D, Rendeiro C, Butler LT, et al. Free Radical Biology & Medicine Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. 2008;45(9):295–305.
7. Murphy T, Dias GP, Thuret S. Effects of diet on brain plasticity in animal and human studies: Mind the gap. Neural Plast. 2014;2014.
8. Neshatdoust S, Saunders C, Castle SM, Vauzour D, Williams C, Butler L, et al. High-flavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: Two randomised, controlled trials. Nutr Heal Aging [Internet].2016;4(1):81–93Availablefrom: . http://www.medra.org/servlet/aliasResolver?alias=iospress&doi=10.3233/NHA-1615
9. Miller MG, Shukitt-hale B. Berry Fruit Enhances Beneficial Signaling in the Brain. J. Agric. Food Chem. 2012;60:5709-5715
10. Riaz A, Khan RA, Algahtani HA. Memory boosting effect of Citrus limon , Pomegranate and their combinations. Pak. J. Pharm. Sci. 2014;27:1837-1840.
11. Rendeiro C, Guerreiro JD, Williams CM, Spencer JP. Postgraduate Symposium. Flavonoids as modulators of memory and learning : molecular interactions resulting in behavioural effects. Proc. Nutr. Soc. 2012;71:246–262.
12. Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R. PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat. Neurosci. 2003;6:136.
13. Kelleher RJ, Govindarajan A, Jung HY, Kang H, Tonegawa S. Translational control by MAPK signalling in long term synaptic plasticity and memory. Cell.2004;116:467-479.
14. Gomez-Pinilla F, Nguyen TT. Natural mood foods. The actions of polyphenols against psychiatric and cognitive disorders. Nutr. Neurosci. 2012;15:127-133.
15. Ma Y, Wang H, Wu H, Wei C, Lee E. Brain derived neurotrophic factor antisense oligonucleotide

impairs memory retention and inhibits long term potentiation in rats. Neuroscience. 1997;82:957-967.
16. Spencer JPE. The impact of fruit flavonoids on memory and cognition. Br J Nutr. 2010;104(SUPPL.3):40–47.
17. Hueston CM, Cryan JF, Nolan YM. Stress and adolescent hippocampal neurogenesis: Diet and exercise as cognitive modulators. Transl Psychiatry [Internet]. 2017;7(4):e1081-17. Available from: http://dx.doi.org/10.1038/tp.2017.48
18. Kim SA, Moore L V, Galuska D, Wright AP, Harris D, Grummer-strawn LM, et al. Morbidity and Mortality Weekly Report (MMWR) Vital Signs : Fruit and Vegetable Intake Among Children — United States , 2003 – 2010. 2015;(September 2015):2003–2010.
19. Peltzer K, Pengpid S. Fruits and vegetables consumption and associated factors among in-school adolescents in five Southeast Asian countries. Int J Environ Res Public Health. 2012;9(10):3575–3587.
20. Institute for Public Health, Ministry of Health Malaysia. Adolescent Nutrition Survey. Kuala Lumpur: Ministry of Health Malaysia; 2017.
21. Augusto RA, Cobayashi F, Cardoso MA. Associations between low consumption of fruits and vegetables and nutritional deficiencies in Brazilian schoolchildren. Public Health Nutr. 2015;18(5):927–35.
22. Spencer SJ, Korosi A, Layй S, Shukitt-Hale B, Barrientos RM. Food for thought: how nutrition impacts cognition and emotion. npj Sci Food [Internet]. 2017;1(1):7. Available from: http://www.nature.com/articles/s41538-017-0008-y.
23. World Health Organization. WHO Guidelines on Drawing Blood for Best Practices in Phlebotomy. Geneva: World Health Organization; 2010.
24. Mohamed K, Tin TS, Jalaludin MY, Al-Sadat N, Majid HA. Comparative validity of a Food Frequency Questionnaire (MyUM Adolescent FFQ) to estimate the habitual dietary intake of adolescents in Malaysia. Asia Pac J Clin Nutr. 2018;27(4):898–907.
25. Shahar S, Safii NS, Manaf ZA, Haron H. Malaysian Atlas of Food Exchanges and Portion Sizes Kuala Lumpur. Kuala Lumpur: MDC; 2015.
26. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia. Malaysian Dietary Guidelines for Children and Adolescents. Putrajaya, Malaysia: Ministry of Health Malaysia;2013.
27. Institute for Public Health, Ministry of Health Malaysia. The National Health and Morbidity Survey, Malaysia School-Based Nutrition Survey 2012. Kuala Lumpur: Ministry of Health Malaysia; 2012.
28. Abdul Ghaffar S, Abdul Talib R, A Karim N. Food choices and diet quality in the school food environment: a qualitative insight from the perspective of adolescents. Mal J Med Health Sci. 2019;15(SP1): 16-24.
29. Ahmad NI, Aam A, A Faris AP, Lee YH, Wan Mohamad WR, Dini Destiani SF. Fruits and vegetable consumption among Malaysian. International Food Research Journal. 2012; 19(3): 1095-1107.
30. Hayati Adilin MA, Holdsworth M, McCullough F, Swift J, Norimah AK. Whole School mapping to investigate the school environment's potential to promote a heathy diet and physical activity in Malaysia. Malaysian Journal of Nutrition. 2015;21(1):1-14.
31. Terry-McElrath YM, O'Malley PM, Delva J, Johnston LD. The school food environment and student body mass index and food consumption. J Adolesc Heal. 2009; 45(3 SUPPL.):S45-56.
32. Antonio M, Giovanna T, Hellas C, Carla R, Fabio G. Nutrition knowledge and other determinants of food intake and lifestyle habits in children and young adolescents living in a rural area of Sicily, South Italy. Public Health Nutrition. 2012; 6(10): 1827–1836.
33. Azahari S, Badari Z, Sc M. The development of a healthy food and nutrient plan for lower-income households in rural areas of Peninsular Malaysia Shamsul Azahari Zainal Badari, M . Sc A thesis in fulfilment of the requirements for the degree of Doctor of Philosophy School of Chemical Engineering Faculty of Engineering. 2014;(January).
34. Ilesanmi OS, Ilesanmi FF, Ijarotimi IT. Determinants of Fruit Consumption among In-school Adolescents in Ibadan, South West Nigeria. Eur J Nutr Food Saf [Internet]. 2014;4(42):100–9. Available from: www.sciencedomain.org
35. Dodd GF, Williams CM, Butler LT, Spencer JPE. Acute effects of flavonoid-rich blueberry on cognitive and vascular function in healthy older adults. 2019;5:119–132.
36. Lamport, D. J., Pal, D., Macready, A. L., Barbosa-Boucas, S., Fletcher, J. M., Williams, C. M., Butler, L. T. The effects of flavanone-rich citrus juice on cognitive function and cerebral blood flow: An acute, randomised, placebo-controlled cross-over trial in healthy, young adults. British Journal of Nutrition, 2016;116(12):2160–2168.
37. Riggs NR, Spruijt-Metz D, Sakuma KL, et al. Executive cognitive function and food intake in children. J Nutr Educ Behav. 2010; 4:398-403.
38. Riggs N, Chou CP, Spruijt-Metz D, et al. Executive cognitive function as a correlate and predictor of child food intake and physical activity. Child Neuropsychol. 2010;16:279-292.
39. Riggs NR, Spruijt-Metz D, Chou CP, et al. Relationships between executive cognitive function and lifetime substance use and obesity related behaviors in fourth grade youth. Child Neuropsychol. 2012;18:1-11.

40. Jaeger BN, Parylak SL, Gage FH. Molecular Aspects of Medicine Mechanisms of dietary fl avonoid action in neuronal function and neuroin fl ammation. [Internet]. Mol Aspects Med; 2018;61:50–62. Available from: . https://doi.org/10.1016/j.mam.2017.11.003
41. Wang H, Zhang Y, Qiao M. Mechanisms of extracellular signal-regulated kinase/cAMP response element-binding protein/brain-derived neurotrophic factor signal transduction pathway in depressive disorder. Neural Regen Res. 2013;8(9): 843-852
42. Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, Greenberg ME. CREB: A Major Mediator of Neuronal Neurotrophin Responses. Neuron. 1997;19:1031-1047.
43. Balkowiee A, Katz DM. Cellular mechanisms regulating activity-dependent release of endogenous brain derived neurotrophic factor from hippocampal neurons. The Journal of Neuroscience. 2002;20(19):7417.
44. Group FSR. USDA Food and nutrient database for dietary studies, 3.3. Beltsville, MD: US Department of Agriculture. Agricultural Research Service. 2018.
45. Kongkachuichai R, Charoensiri R, Sungpuag P. Carotenoid, flavonoid profiles and dietary fiber contents of fruits commonly consumed in Thailand. Int. J. Food Sci. Nutr. 2010; 61: 536–548.
46. Yahiya EM, Alexandria VA. Handling tropical fruits. Scientist Speak: World Foods Logistics Organization; 2006.
47. Park YS, Cvikrova M, Martincova O, et al. In vitro antixidative and binding properties of phenolics in traditional, citrus and exotic fruits. Food Res Int. 2015; 74: 37-47.
48. Bazzano LA, He J, Ogden LG. Fruit and vegetable intake and risk of cardiovascular disease in us adults: the first national health and nutrition examination survey epidemiologic follow-up study. The American Journal of Clinical Nutrition. 2002; 76: 93–99.
49. Lakshmi A, Kaul P. Nutritional potential, bioaccessibility of minerals and functionality of watermelon (citrullus vulgaris) seeds. Food Science and Technology. 2011; 44:1821–1826.
50. Pochwat B, Sowa-Kucma M, Kotarska K, Misztak P, Nowak G, Szewczyk B. Antidepressant-like activity of magnesium in the olfactory bulbectomy model is associated with the AMPA/BDNF pathway. Psychopharmacology (Berl). 2015; 232: 355-67.