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PUTRA-Adol study: protocol for an observational follow-up study to assess the tracking of dietary patterns linked to cardiometabolic risk factors and its prospective relationship with non-alcoholic fatty liver disease, carotid intima-medial thickness and mental well-being during adolescence in Malaysia

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ABSTRACT

Introduction Growing evidence suggesting that dietary intakes of adolescents are generally of poor quality but not adequately assessed in relation to the early manifestation of non-communicable diseases. This study aimed; (1) to examine tracking of an empirical dietary pattern (DP) linked to cardiometabolic risk factors and, (2) to assess prospective relationships between a DP characterised by high intakes of dietary energy density (DED) and added sugar, and cardiometabolic risk factors, non-alcoholic fatty liver disease (NAFLD), carotid intima-medial thickness (CIMT) and mental well-being during adolescence.

Methods and analysis The PUTRA-Adol is a prospective follow-up study that builds up from 933 Malaysian adolescents who were initially recruited from three southern states in Peninsular Malaysia in 2016 (aged 13 years then). Two sessions are planned; the first session will involve the collection of socio-economy, physical activity, dietary intakes, mental well-being, body image, risk taking behaviour, sun exposure, family functioning and menstrual (in women) information. The second session of data collection will be focused on direct assessments such as venesection for blood biochemistry, anthropometry and ultrasonography imaging of liver and bilateral carotid arteries. Z-scores for an empirical DP will be identified at 16 years using reduced rank regression. Multilevel modelling will be conducted to assess the tracking of DP and prospective analysis between the DP, cardiometabolic health, NAFLD, CIMT and mental well-being.

Ethics and dissemination Ethical approval for the conduct of this follow-up study was obtained from the Universiti Putra Malaysia's Ethics Committee for Research Involving Human Subjects (JKEUPM) (Reference number:

Strengths and limitations of this study

► Understanding the tracking or maintenance of dietary patterns may be useful in identifying sub-groups of population and timing for effective interventions to improve diet quality during adolescence.

► One of the first studies in Malaysia to prospectively assess associations between an empirically identified dietary pattern and various cardiometabolic risk factors, non-alcoholic fatty liver disease (NAFLD), risk of atherogenesis and mental well-being during adolescence.

► Collects various prospective data on predictors of cardiometabolic risk factors, NAFLD, risk of atherogenesis and mental well-being during adolescence.

► Self-report of various predictor variables may be associated with recall bias.

► The generalisation of the study findings may be limited due to the location of the study samples.

JKEUPM-2019–267). The findings from this study will be disseminated in conferences and peer-reviewed journals.

Discussion The findings gathered from this study will provide evidence on prospective relationships between DPs, cardiometabolic risk factors, NAFLD, early atherosclerosis and mental well-being and that it may be mediated particularly DED and added sugar during adolescence.

BACKGROUND

The concept of tracking in epidemiological studies refers to the stability of

measurements on an individual over time.[1] [2] Understanding the tracking of specific childhood dietary patterns that are predictive of adult's health outcomes may provide imperative information for an early prevention of diet-related chronic disease risk factors. This is of much importance considering that growing evidence suggests a link between diet and lifestyle factors in childhood and adolescence and the risk of developing diet-related diseases in adulthood.

Several observational studies conducted in Western countries have evaluated the tracking of nutrient or single food group intakes as well as dietary patterns (DP) between childhood and adulthood.[3–6] However, to the very best of our knowledge, no prospective studies have examined the tracking of empirical DP that summarise total dietary intake among adolescents in Malaysia. To help identify targets for interventions to improve DP in adolescents, information on DP tracking is needed. Furthermore, the understanding of the food groups within the pattern that shows stronger or weaker tracking, can help identify dietary targets that may be more or less acquiescent to change.

The formation of lifelong dietary habits may be evident in adolescents due to the rapid physiological and psychological development as well as the increasing autonomy and independence during this stage of life. In addition, adolescents who often consumed dietary intakes of poor quality were found to maintain these dietary habits into adulthood where the manifestation of various comorbidities took place.[7] [8] We previously identified a 'high sugar, high fibre, high energy dense and low fat' DP among adolescents aged 13 years recruited from a few public secondary schools in three southern states of Peninsular Malaysia.[9] The identified DP was positively associated with dyslipidaemia and individual markers of cardiovascular diseases (CVD) including total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) in both male and female adolescents.[9] This may be explained by the distinctive nutrient profile of the 'energy dense, high sugar, high fibre and low fat' DP. Based on evidence from adults' studies, foods high in sugar and energy dense are probably the most important dietary characteristic for the development of diseases associated with metabolic abnormality in young people.[10] While data on the consumption of added sugar, particularly of those sugar-sweetened beverages (SSBs) is somewhat not that exhaustive in Malaysia, higher consumption of SSBs are linked to obesity and diabetes in low and middle income countries.[11] Considerable evidence highlighted that different types of disaccharides play a role in influencing metabolic complications; glucose increases dietary glycaemic load while fructose elevates abdominal obesity.[12] These effects appear partly but not entirely mediated by adiposity, consistent with independent adverse effects of SSBs on other pathways such as hepatic de novo lipogenesis, visceral fat accumulation and uric acid production.[13] In addition to the direct harms, SSBs may increase cardiometabolic risk by displacing other healthier foods in the diet, for example,

water, fruits, vegetables and milk and a greater intake of processed foods.[14]

In children and adolescents, only a few studies have reported prospective associations between DP and cardiometabolic risk factors. Among these studies, only one prospective study has investigated the relationship between an empirically derived 'energy dense, high fat and low fibre' DP and cardiometabolic risk factors, namely, overall metabolic risk factors, insulin resistance, glucose and waist circumference among adolescents in Western Australia.[4] In this similar study cohort, a higher adherence to a 'Western' DP derived using factor analysis at age 14 years was significantly associated with the risk of developing non-alcoholic fatty liver disease (NAFLD) at age 17 years.[15] In the Cardiovascular Risk in Young Finns Study, a 'traditional' DP, characterised by high intakes of rye, potatoes, butter, sausages, milk and coffee using principal component analysis, was positively associated with TC, LDL-C, apolipoprotein B and C reactive protein concentrations in both women and men, as well as with systolic blood pressure and insulin concentrations specifically in women, after a 21-year follow-up.[16] Furthermore, findings from the Northern Ireland Young Hearts Project found that adolescents aged between 12 and 15 years who adhered to a 'Western' DP characterised by high intakes of soft drinks, crisps and chips were prospectively associated with elevated homocysteine levels at 20–25 years of age.[17] As studies assessing the prospective relationship between specific DP and cardiovascular risk factors are largely limited to Western countries, data from a local setting is important for early identification and understanding of the associated determinants and the most effective timing for interventions.

Since 24% of our study adolescents were found to have dyslipidaemia as well as positive associations between the identified 'high sugar, high fibre, high energy dense and low fat' DP and dyslipidaemia, it is worthwhile to examine its prospective relationship with other metabolic abnormalities such as NAFLD and markers of atherosclerosis.[9] While there is no set definition for dyslipidaemia in adolescents, it is commonly addressed when one or more of the lipid parameters elevates, that is, TC, triglycerides (TG), LDL-C and low levels of high-density lipoprotein-cholesterol (HDL-C). Subjects with dyslipidaemia are often linked to NAFLD and CVD.[18] This physiological dysfunction is directly related with the risk of atherogenesis and subsequently predisposing subjects to CVD. Apart from that, we also found that the identified 'high sugar, high fibre, high energy dense and low fat' DP was positively associated with poorer life satisfaction scores in these adolescents.[19] It was found that the increasing trend of cardiometabolic risk factors is parallel to the mental health problems among Malaysian young people over the last decade.[20–22] As such, it may be possible that the determinants of cardiometabolic risk factors, especially the dietary predictors, may also influence the state of mental health in adolescents. For instance, the intake of unhealthy DP, characterised by high intake of takeaways,

confectionary and red meat, was prospectively associated with poor mental health status among adolescents aged 14 years in Western Australia.[15] However, to the best of our knowledge, no studies have explored the prospective associations between empirical DPs and dyslipidaemia, NAFLD and markers of early atherosclerosis as well as mental well-being during adolescence in Malaysia.

AIMS

The aims of this study are; (1) to examine the tracking of a DP characterised by high intakes of dietary energy density (DED) and added sugar that was previously linked to cardiometabolic risk factors and, (2) to assess prospective relationships between the DP characterised by high intakes of DED and added sugar and cardiometabolic risk factors, NAFLD, risk of atherogenesis using carotid intima-medial thickness (CIMT) information and mental well-being during adolescence.

HYPOTHESES

The following hypotheses will be tested:

► A dietary pattern characterised by high intake in percentage of energy from sugar, fibre density, dietary energy density and low percentage of energy from total fat will feature at age 16 years.
► The identified 'high sugar, high fibre, high energy dense and low fat' DP will strongly track between 13 and 16 years of age.
► Greater adherence to the identified 'high sugar, high fibre, high energy dense and low fat' DP between 13 and 16 years of age will be prospectively associated with unfavourable cardiometabolic risk factors, NAFLD and markers of atherogenesis.
► The identified 'high sugar, high fibre, high energy dense and low fat' DP will be prospectively associated with poorer mental well-being between 13 and 16 years of age.

METHODS AND ANALYSIS
Study settings

The PUTRA-Adol is a prospective follow-up study that builds up on a total of 933 Malaysian school-going adolescents (633 women and 300 men) who were initially recruited from 21 public secondary schools in three southern states of Peninsular Malaysia namely Negeri Sembilan, Melaka and Johor in 2016. The details of the study adolescents whose age was 13 years at their recruitment in 2016 were previously described.[9] This study is funded by the Ministry of Higher Education, Malaysia, and currently being coordinated by the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM). Ethical approval for the conduct of this follow-up study was obtained from the UPM's Ethics Committee for Research Involving Human Subjects (JKEUPM) (Reference number: JKEUPM-2019–267).
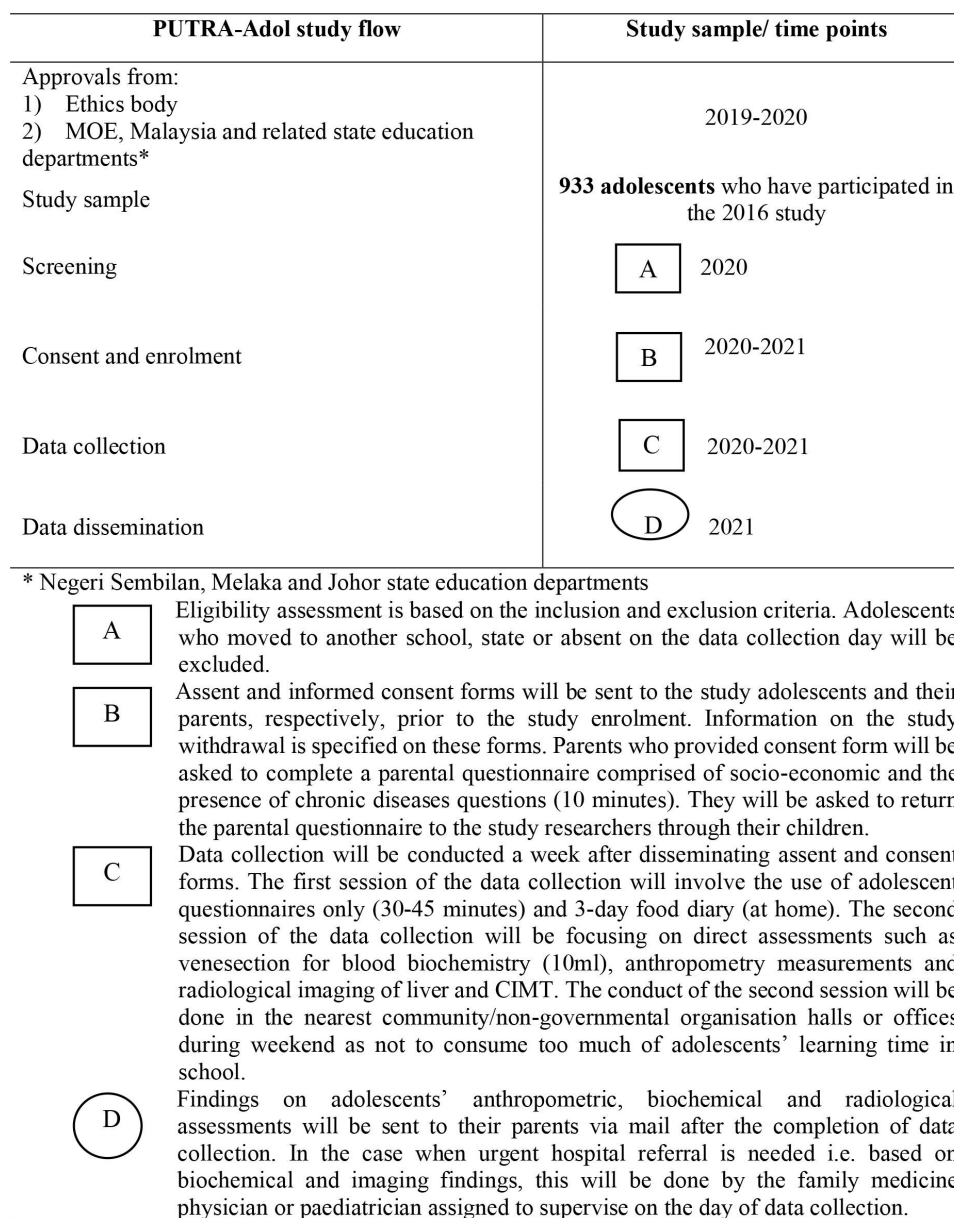
Recruitment and consent

Prior to the study conduct, written approvals from the Ministry of Education (MOE), Malaysia, and each state education department will be obtained. Following these approvals, 21 schools that participated in 2016 will be contacted for the current follow-up study arrangement, that is, a suitable date and time for the study to take place. Adolescents (n=933) who participated in the initial study will be invited to participate. They will be screened once again for the presence of physical limitations and asked to self-report any chronic diseases such as diabetes mellitus, heart disease, asthma, renal dysfunction and other metabolic disorders. Adolescents who either reported any of the above-mentioned diseases or were found to have physical limitations will be excluded from this follow-up. In addition, adolescents for whom the follow-up is impractical, for example, moved to another school or state or absent on the day of data collection, will be excluded. A contemporaneous Malaysian study among adolescents in 2016 reported an attrition rate of 30%; as such, we would expect a similar proportion of adolescents not to be available in the current study (n=280).[20] With this number of adolescents' recruitment, the current study will be powered up to 73% based on the expected changes to be observed in the waist circumference measurement (one of the main outcome of the study).[23] To be eligible to participate in the follow-up study, adolescents must meet the following criteria: (1) have participated in the previous 2016 study,[9] (2) read and speak Malay or English fluently, (3) able to follow the research procedures, that is, venesection for biochemical assessments and ultrasonography imaging of liver and CIMT and (4) provide assent and informed consent. Eligible adolescents who agree to participate in this study will be given an information sheet on the study and asked to sign an assent form afterwards. In addition to that, an invitation letter and an informed consent form will be sent to the eligible parents illustrating the study details and recruitment process. Adolescents whose parents have agreed to participate will be enrolled in this study a week after disseminating the information sheet and consent form to the parents. Parents who do not wish their children to participate in this study will be respected. A graphical summary of the study flow is shown in figure 1.

Data collection

Data collection is planned from August 2020 to April 2021 with a total of two sessions for each participating school (figure 2). During the first session at school, study enumerators will describe the nature of the study, including venesection for biochemical assessments and ultrasonography procedures as well as some details pertaining to the adolescent questionnaires. Adolescents will be also informed that any unforeseen outcomes from the study procedures will be made available to their parents, subsequently.

Soon after the thorough description on the study, the enrolled study adolescents will be asked to self-administer

PUTRA-Adol study flow	Study sample/ time points
Approvals from: 1) Ethics body 2) MOE, Malaysia and related state education departments*	2019-2020
Study sample	**933 adolescents** who have participated in the 2016 study
Screening	A 2020
Consent and enrolment	B 2020-2021
Data collection	C 2020-2021
Data dissemination	D 2021

* Negeri Sembilan, Melaka and Johor state education departments

A	Eligibility assessment is based on the inclusion and exclusion criteria. Adolescents who moved to another school, state or absent on the data collection day will be excluded.
B	Assent and informed consent forms will be sent to the study adolescents and their parents, respectively, prior to the study enrolment. Information on the study withdrawal is specified on these forms. Parents who provided consent form will be asked to complete a parental questionnaire comprised of socio-economic and the presence of chronic diseases questions (10 minutes). They will be asked to return the parental questionnaire to the study researchers through their children.
C	Data collection will be conducted a week after disseminating assent and consent forms. The first session of the data collection will involve the use of adolescent questionnaires only (30-45 minutes) and 3-day food diary (at home). The second session of the data collection will be focusing on direct assessments such as venesection for blood biochemistry (10ml), anthropometry measurements and radiological imaging of liver and CIMT. The conduct of the second session will be done in the nearest community/non-governmental organisation halls or offices during weekend as not to consume too much of adolescents' learning time in school.
D	Findings on adolescents' anthropometric, biochemical and radiological assessments will be sent to their parents via mail after the completion of data collection. In the case when urgent hospital referral is needed i.e. based on biochemical and imaging findings, this will be done by the family medicine physician or paediatrician assigned to supervise on the day of data collection.

Figure 1 Flow of the PUTRA-Adol study. CIMT, carotid intima-medial thickness; MOE, Ministry of Education.

a set of questionnaires on socio-economy, physical activity, dietary intakes (food frequency questionnaire (FFQ)), mental well-being, body image, risk taking behaviour, sun exposure, family functioning and menstrual information (in women) at school as well as at home (3-day food diary). The study adolescents will be requested to return the 3-day food diary and their parents' questionnaires during the second session of the data collection.

The second session of the data collection will be conducted elsewhere, that is, community/non-governmental organisation halls or offices, and it will be planned over weekends as not to consume too much of the adolescents' learning time in school. Since the second session comprised of direct assessments, that is, anthropometric measurements, venesection for biochemical assessments and ultrasonography imaging, this visit will be attended by a group of healthcare professionals including a family medicine physician or paediatrician, radiologist and research enumerators. The research enumerators will coordinate the study arrangement, collect and check parental questionnaires and 3-day food diaries for their completeness and conduct anthropometric measurements. Blood pressure and venesection will be performed by family medicine physician or paediatricians and phlebotomists, respectively. Study adolescents will be reminded to fast overnight (at least for 8 hours) during the first session as well as a day before the second session via WhatsApp messages. An emergency standard measure will be employed by the assigned family medicine physician or paediatrician if there are any complications during the venesection procedure, that is, fainting, nausea or convulsion. Adolescents who experience any complications after the venesection procedure such as infection, prolonged

1st session at school	At participants' home	2nd session at community/ non-governmental organisation halls or offices
- Briefing on the follow-up study - Screening of adolescents based on inclusion and exclusion criteria - Interested adolescents will be asked to sign an assent form - Consent form, parental questionnaire and 3-day food diary will be given to the eligible adolescents - Eligible students will be asked to complete a set of questionnaires in school	- Participants will be requested to inform about the study to their parents - Interested parents will be asked to sign informed consent form given and complete the parental questionnaire - Adolescents will be asked to fill up the 3 days food diary at home - Participants will be asked to fast overnight a day before the second session	- Parental informed consent form and questionnaire will be collected - Dietary intake data will be checked and verified for completeness before submission - Anthropometry and clinical data i.e. blood pressure, venesection for blood biochemistry and ultrasonography imaging will be done

Figure 2 The process of data collection in the PUTRA-Adol study.

bleeding, excessive bruising and pain, will be asked to immediately contact the nearest health care clinic for further medical care. Ultrasonography imaging will be performed by an experienced radiologist in a closed room. Any incidental findings from the ultrasonography imaging assessment such as pregnancy or tumour will be immediately referred to the nearest hospital for further diagnosis confirmation. Such cases will be also informed to the adolescents' parents.

Adolescents who were enrolled in this follow-up study will receive a small appreciation token during the data collection days such as snack foods, that is, bread/bun, and drinks. In addition to that, verified study findings including biochemical, imaging and other screening assessments for mental health (depression), body image disorder and high risk behaviour (smoking and drug intake) measured in this study will be made available to the study adolescents and their parents soon after the data collection. Along with the study findings, a referral letter for further expert management will be sent to the parents if the test results are found to be unfavourable. These results will not be revealed to the school authorities to protect data confidentiality of the study adolescents.

Outcome measures

Several outcome measures, namely, cardiometabolic risk factors, NAFLD, CIMT, life satisfaction and subjective mental well-being, will be investigated in this follow-up study.

Cardiometabolic risk factors

Findings from both the anthropometric and biochemical assessments will be used to determine cardiometabolic risk factors in this study. It will include overweight/obesity, abdominal obesity, blood pressure, lipid profile, fasting glucose and insulin. Anthropometric assessments including body weight (kg), height (m) and waist circumference (cm) measurements will be conducted. Body weight will be measured to the nearest 0.1 kg with a seca 213 portable stadiometer (seca, Germany). Participants will be measured in light clothing and no shoes. Height will be measured in cm, to the nearest 0.1 cm with a seca 213 portable stadiometer (seca, Germany) from the adolescent's head to toe in an upright standing position with the body touching the wall. Waist circumference will be measured to the nearest 0.1 cm at the midpoint between the lower border of the ribs and the upper border of the pelvis using a seca 201 Ergonomic Measuring Tape (seca, Germany). All measurements will be measured twice, and each mean value of the measurements will be computed and used in the analysis.

On measuring weight and height, body mass index (BMI) (kg/m^2) will be estimated, whereby weight (kg) will be divided with height (m) squared. BMI z-score for age and sex will be calculated using the WHO Anthro Software V.3.2.2.[24] Using BMI z-scores of more than one and two SD above the WHO growth standard median, accordingly, overweight and obesity will be classified. Z-scores for waist circumference will be computed and abdominal obesity will be defined according to the Malaysian waist

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circumference centile curves of equal or more than the 90th centiles.[25]

Resting blood pressure will be assessed using an automated blood pressure monitor (Omron, Omron Healthcare, Illinois, USA). Two consecutive blood pressure measurements separated by 30 s intervals will be made in a seated position on the left arm. The average of the two systolic and diastolic blood pressure measurements will be computed. Since the assessment of blood pressure in this study will be done merely as a screening purpose, adolescents whose systolic and diastolic readings exceed 120 mm Hg and 80 mm Hg, respectively, will be referred to the nearest health clinic for further diagnosis and management.[26]

A total of 10 mL of blood will be withdrawn from each adolescent after an overnight fast. The blood samples will be stored in a cool box (4°C) before transporting it to a certified single laboratory company for the analysis of fasting insulin, blood glucose and lipid profile (TC, TG and HDL-C). In addition, insulin resistance will be estimated using homoeostasis model assessment (HOMA) by Matthews *et al* while LDL-C will be calculated using Friedewald *et al*'s 1972 formula.[27 28] Dyslipidaemia will be defined when any of the lipid parameters are not within the cut-off value; cholesterol ≥5.2 mmol/L, HDL-C ≤1.0 mmol/L, LDL-C ≥3.4 mmol/L and TG ≥1.5 mmol/L. Apart from estimating dyslipidaemia, lipid parameters will be individually defined using the abovementioned cut-offs. High blood glucose and insulin levels will be defined when blood glucose ≥5.6 mmol/L and serum insulin ≥25 uIU/mL, respectively. Insulin resistance will be defined when HOMA value ≥2.5 unit.[29] In view of the fact that there is a lack of consensus on the definition of metabolic syndrome (MetS) in adolescents, the clustering of cardiometabolic risk factors (≥3 risk factors, including blood pressure and abdominal obesity) will be considered for further analysis in this study.[30]

Serum will be aliquot and stored at −80°C temperature for future research usage.

NAFLD and CIMT

Ultrasonography of the liver and bilateral carotid arteries will be conducted by an experienced radiologist using the modified scan protocol by Kim *et al* in 2009.[31] By using a Mindray diagnostic ultrasound machine and a 2–5-MHz curved array probe, ultrasound of the abdomen focusing on the liver will be performed in a high-resolution B-mode setting. Adolescents will be asked to fast (at least 8 hours prior) before the ultrasonography imaging of the liver is done. The imaging of the liver will include both long-axis and transverse views. Liver span measurement will be performed on longitudinal images. By assessing each of the eight Couinaud segments of the liver, the liver parenchyma will be evaluated for focal and/or diffuse abnormalities. Apart from that, the echogenicity of the liver will be compared with that of the right kidney and the spleen to diagnose the presence of NAFLD. If the echogenicity is increased compared with the spleen, the

following classification will be used to grade the severity of NAFLD based on Di Martino *et al*[32]:

► Mild NAFLD: echogenicity of the liver parenchyma is increased compared with the spleen.
► Moderate NAFLD: the echogenicity of the walls of the portal vein branches is lost or obscured by the increased liver echogenicity.
► Severe NAFLD: the echogenicity of the right hemidiaphragm is lost/ obscured.

CIMT will be measured by using a linear ultrasound probe with 7.5 MHz.[31] The adolescent will be positioned semi-recumbent, with the neck extended and rotated away from the side being evaluated. Imaging will be performed on the right and left carotid arteries using two-dimensional grey scale imaging in axial and longitudinal views. By identification of the near wall (closest to the skin surface) and the far wall (farthest from the skin surface) of the three arterial segments: the common carotid artery[25] located within the carotid triangle, and the carotid bifurcation beginning at the tip of the flow divider (site of the division of flow between the external carotid artery and internal carotid artery) and extending 8–10 mm proximally, the measurements of the CIMT will be made. The CIMT will be measured as the distance between the echogenic lines of the far wall of the bilateral common carotid arteries (CCAs), approximately 1 cm proximal to the carotid bulb. The mean maximal CIMT will be defined as the mean of two measurements taken by a single reader of each CCA. If there was the presence of posterior wall plaque, this will be included in the measurements. The mean value of the two maximal CIMT measurements (mean maximal CIMT) will be used as the outcome measure. This mean maximum CIMT has demonstrated good reproducibility (intra-observer coefficient of variation of 2.1%).[31] The diagnosis of NAFLD and CCA atherosclerosis will be made by the radiologist in this study. It is estimated that the ultrasound imaging procedures will take 30 min per subject.

Mental well-being

Mental well-being of the study adolescents will be assessed using two instruments namely the Multidimensional Students' Life Satisfaction Scale (MSLSS) and Depression Anxiety Stress Scale (DASS-21).[33–35] The original MSLSS was developed by Huebner with 40-items to measure adolescents' life satisfaction within five domains (family, friends, school, living environment and self-life satisfaction). This instrument was later shortened by Sawatzky *et al* in 2009 to 18-items by removing all the negatively worded and some positively worded items. Adolescents in this study will be asked to respond to their thoughts on life experiences during the past several weeks. Six-point response scale will be used ranging from 1=strongly disagree to 6=strongly agree. Total score will be computed with a high score that corresponds to a high level of overall satisfaction and specific domains of life satisfaction. A 21-item self-reporting DASS scale, developed by Lovibond will be used to assess subjective depressive

and anxiety symptoms.[35] DASS-21 is a set of three self-report scales designed to measure the emotional states of depression, anxiety and stress. Each of the three DASS-21 scales contains seven items, divided into subscales with similar content. DASS-21 in Malay language has been validated and showed high internal consistency.[36] Scores on the DASS-21 will be multiplied by 2 to calculate the final score.

Predictor measures

A various number of predictor measures that may be associated with the outcome measures will be examined in this study. These predictor measures incorporate both parental and adolescent factors.

Socio-economic factors

Adolescents enrolled in this study will be asked to complete questions on socio-economic factors including gender, ethnicity, religion, date of birth and their school location. Parental questionnaires on socio-economic factors will be completed at home and this will be comprised of age, education attainment, household income (RM), occupation and number of dependents. Self-reported weight and height, smoking status and the presence of chronic diseases such as diabetes, hypertension, hypercholesterolaemia, cancer, heart disease and osteoporosis in parents and their relatives will also be asked in the parental questionnaire.

Physical activity

Self-reported physical activity level will be assessed using a validated physical activity questionnaire for adolescents.[37 38] This questionnaire comprises of nine items and it will assess the physical activity levels of the study adolescents in the past 7 days. The first item will include the type and frequency of sports or/and dances performed in the past 7 days. The second to eighth items will assess activities performed during physical education classes, recess, lunch, after school, evenings, weekends and leisure periods. Item eight will include the frequency for physical activity level from the previous week and item nine will assess any unusual activities from the previous week. This questionnaire was previously validated and showed good internal consistency and acceptable validity.[38] However, one of the main limitations of this questionnaire is that it do not provide details on intensity and therefore, differences between moderate and vigorous activities cannot be estimated.

Body image assessment

The Contour Drawing Figure Rating Scale (CDFRS) will be used to assess body image among adolescents in this study.[39] CDFRS consists of nine line-drawings of women's and men's bodies arranged from the smallest to largest. Both male and female adolescents in the present study will be asked to draw a mark below the drawing that they think most closely approximates their current body size.

Family functioning assessment

Family functioning will be assessed using validated six-positive items from the General Functioning subscale (GF6+) in the McMaster Family Assessment Device (FAD).[40] The GF6+ was found to have similar psychometric properties and able to identify healthy and unhealthy levels of family functioning as per the FAD.[40] The score for each item ranges from 1 (best functioning) to 4 (worse functioning). The total score is the sum of the values of each item divided by the number of items on the subscale. The score below 2 indicates healthy family functioning, while a score of 2 and above indicates unhealthy family functioning.

Risk-taking behaviours

Three risk taking behaviours, that is, internet usage, smoking and drug use, will be particularly assessed in this study. The majority of these risk-taking behaviour questions will be adapted from the Adolescent Healthy Survey conducted in 2017 by the Institute for Public Health, Malaysia. There will be a total of eight questions on internet usage among adolescents. Questions 1–8 will be on the internet access using any internet connecting devices in the past 30 days as well as using the internet as a learning source. Additional questions on internet addiction will also be measured using a self-administered 20-items Malay Version Internet Addiction Test questionnaire.[41] This instrument consists of 5-point Likert scales (never to very frequent) and adolescents whose score is equal or greater than 43 will be defined as having internet addiction.

There will be 10 questions related to smoking history and the type of tobacco products used in the past 30 days, for example, manufactured cigarette, e-cigarette/vape, roll-your-own cigarette, traditional hand-rolled cigarette, shisha, cigar or pipe. Meanwhile, there will be seven questions related to drug use and the study adolescents will be asked to indicate the type of drugs commonly used (heroin, morphine, glue, amphetamine or methamphetamine (ecstasy, syabu, ice), marijuana/ganja (except prescribed medicine)) in the past 30 days and report the history of drug taking.

Sun exposure

Adolescents' sun exposure will be assessed using Measures of Sun Exposure and Sun Protection Practices questionnaire.[42] A total of seven questions will be asked to assess the sun exposure; questions numbers 1–5 measure outdoor activities and sun protection practices, while the remaining two questions will be on the intake of vitamin D supplementation and skin pigmentation types, respectively.

Menstruation information

Questions related to menstruation information will be asked to female adolescents and this will be done using a questionnaire by Parker et al.[43] A total of eight questions

will assess adolescents' history on the menstrual cycle and known medical conditions related to menstrual disorders.

Dietary assessment

Adolescents in this study will be asked to provide information on dietary intake using a validated adolescent FFQ, which was used in the previous study[9] as well as a 3-day food diary (two weekdays and one weekend day). The study adolescents will be asked to complete the 3-day food diary at home with parental assistance. Guidance for completing the 3-day food diary will be provided to each study adolescent during the first session of data collection in school. For instance, adolescents will be asked to provide detailed information on food and drinks consumed, including brand name (where available) and quantity consumed based on household measures or actual weights from labels or packets. Apart from this, adolescents will be asked to record any eating outs, fast food consumption and night eating in the food diary. Illustrated flip charts containing local foods and a measurement cup/bowl/spoon will be used as supplementary tools to assist the study adolescents to estimate the portion size of foods they consumed when completing the FFQ in school. Foods and beverages from the 3-day food diary will be coded using NutriPro software. The reported food items will be categorised into 14 predefined food groups based on their own nutrient profiles or culinary usage, and their hypothesised contribution to cardiometabolic diseases.

Dietary misreporting is a very common issue among adolescents. Therefore, estimation of dietary misreporting using a standardised equation will be performed.[44] This equation will be based on energy intake to total energy expenditure ratio and its 95% confidence limit cut-offs will represent dietary misreporting levels. The variable of dietary misreporting will be comprised of underreporting, plausible and over-reporting and will be used as a potential covariate in the prospective modelling.

Dietary patterns

The analysis of DP will be performed using reduced rank regression (RRR). The RRR approach identifies a linear combinations of weighted food intake, that is, food groups by explaining the maximum variation in a set of response variables, that is, biomarkers or nutrients that are hypothesised to be linked to health outcomes. The identification of the RRR DP in these study adolescents was published previously.[9] In brief, 14 predefined food groups and three dietary risk factors for higher cardiometabolic risks, that is, DED, fibre density and percentage of energy from total fat and added sugar will be used as predictors and response variables, respectively, in the RRR analysis. These response variables were linked to obesity and cardiometabolic risk factors in children and adolescents from birth cohorts such as the Avon Longitudinal Study of Parents and Children in the UK and the Western Australian Pregnancy (Raine) cohort study.[4 5 45–47] The food groups will be estimated in gram (g) intakes while DED and fibre density

will be calculated by dividing energy intake in kilojoules (kJ) by weight in g for the total daily food consumed (without beverages) and absolute fibre intake in g by total daily energy intake in megajoules (MJ), accordingly. The percentage of energy from total fat will be estimated by dividing the total energy intake from total fat in kJ by the total energy intake in kJ and subsequently multiplied by 100. This will similarly be done for the percentage of energy from added sugar.[47] Each adolescent in this study will receive a z-score for the identified DP and the z-zcore will be used in multilevel modelling for establishing associations between adherence to the DP, cardiometabolic risk factors, NAFLD, CIMT and mental well-being in this study. Separate RRR analysis will be performed for male and female adolescents due to sexual dimorphisms and differences in dietary intake during adolescence.[48]

Data analysis

Predictor variables including adolescents and their parents' socio-economic characteristics, physical activity, dietary intakes, the identified empirical dietary pattern, body image, risk taking behaviours, sun exposure, family functioning and menstrual information (in female adolescents) will be summarised, accordingly. Normally distributed continuous variables will be described using means and SD and non-normally distributed continuous variables will be reported using medians and IQRs. Percentages (%) for categorical data will be also reported. In addition, means and SD or medians and IQRs for outcome variables, that is, anthropometric, biochemical, clinical measurements as well as life satisfaction and depressive and anxiety scores will be reported. Following this, the prevalence (%) of overweight and obesity, abdominal obesity, unfavourable cardiometabolic risk factors, the presence of NAFLD and markers of atherogenesis, poor life satisfaction, depression and anxiety will be described.

Univariate and bivariate analyses and group comparisons between male and female adolescents particularly, will be performed by means of X^2 test, t-tests or equivalent non-parametric Mann-Whitney U test, and Pearson or Spearman correlation coefficients.

RRR analysis will be performed to identify a 'high sugar, high fibre, high energy dense and low fat' DP. This DP was previously identified in these adolescents in 2016.[9] The identification of a similar DP will facilitate the tracking analysis between 13 and 16 years of age. Tracking coefficients will be assessed using an approach recommended by Twisk in 2003.[49] This will be done by regressing DP z-scores identified at 13 years on DP z-scores measured at 16 years using the generalised estimating equation. By doing so, the longitudinal association between the DP found at 13 and 16 years will be modelled. Tracking coefficients range between 0 and 1 with tracking coefficients ≥0.40 will be considered as moderate tracking.[5]

Characteristics of predictor variables including dietary intake and DP characterised by food high in sugar, DED, fibre and low in fat (as previously identified) and its key contributing food groups will be presented.

Outcome variables will be tabulated across the quartiles of DP z-scores to understand the trend of any associated factors. One-way analysis of variance or non-parametric Kruskal-Wallis test will be conducted to analyse differences between predictor or outcome variables, and quartiles of DP z-scores. Subsequently, prospective multilevel modelling will be conducted to examine associations between the identified DP and various cardiometabolic risk factors, NAFLD, marker of atherogenesis and mental well-being. These prospective modelling will be adjusted for various potential covariates including age, maternal education, dietary misreporting, the location of school (urban or rural), physical activity and BMI (for biochemical parameters). Separate analysis will be run in male and female adolescents due to puberty-related differences and sex dimorphism.[50]

DP will be analysed using SAS V.9.1, while other analyses will be conducted using Stata V.12 (StataCorp, College Station, Texas). All statistical tests will be considered significant at a $p < 0.05$ level.

Patient and public involvement

No patients and public were involved in the development of the research concept, outcome measures or design of the study.

DISCUSSION

The findings gathered from this study will further enhance evidence on the prospective relationships between DP, cardiometabolic risk factors, NAFLD, markers of atherogenesis and mental well-being and that these relationships may be mediated in part by selected nutrients, particularly dietary energy density and percentage energy from added sugar during adolescence.[9 47] The possible direct and independent influences of these specific nutrients on cardiometabolic function and mental well-being will highlight the importance of interventions and nutrition-related programmes to prevent future CVD and mental health issues. However, due to the lack of cohort studies in children and adolescents in Malaysia, longitudinal analyses assessing the contribution of poor DP on cardiometabolic risk factors and the manifestation of NAFLD, atherogenesis markers and mental health issues were therefore limited. Furthermore, the assessment of DP tracking in this study will provide information on the most appropriate time point for effective interventions to take place during the adolescence stage.

This follow-up study is particularly important as a national survey in Malaysia in 2017 has reported that a majority of adolescents were found to consume dietary intakes of poor quality, that is, higher consumption of SSBs and fast food, and low intakes of fruit and vegetables.[51] Furthermore, this study will contribute to the quantitative findings on the health and well-being of children in Malaysia as desired by the Malaysian Education Blueprint 2013–2025. Globally, this study is relevant with the third goal of Sustainable Development Goals, whereby the good health and well-being of children should be protected. As Malaysia proceeds with a great acceleration towards a developed economy status, it is expected that the population's lifestyle will continue to change. Therefore, the present policies and strategies should be improved with sustainable, comprehensive and multifaceted preventive actions to promote healthy nutritional exposure early in life.

The findings of this study are expected to corroborate to those reported in the Western world and subsequently highlight the importance of healthy dietary intake early in life. Proper nutrition in early life offers one of the most effective and least costly ways to decrease the burden of chronic and non-communicable diseases and their risk factors. By identifying the dietary risk factors for cardiometabolic and mental health, this research will have a major positive impact on the lives and prospects of adolescents in the later stages of life.

Ethics approval and consent to participate

Ethical approval for the conduct of this follow-up study was sought from the UPM's Ethics Committee for Research Involving Human Subjects (JKEUPM) (Reference number: JKEUPM-2019–267). Prior to the study conduct, written approvals from the MOE, Malaysia, and each state education department will be obtained. The study respondents and their parents will be asked to provide written consent before the commencement of the study.

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