ISSN2434-2688

Asian Journal of Dietetics

Vol.2 No.1, 2020

AJD

ASIAN JOURNAL OF DIETETICS

Official journal of the Asian Federation of Dietetic Associations (AFDA)

ISSN2434-2688

Asian Journal of Dietetics

Vol.2 No.1 2020

Contents

ORIGINAL

Nutritional Care of Preterm Infants: from Global Guidelines to Local Practice

Shih-Ching Lo[1, 2] *, Hsing-Chun Lin[1, 2], Cheau-Feng Lin[3, 4]

[1]*Department of Nutrition, Chung Shan Medical University Hospital, Taiwan, R.O.C*
[2]*Department of Nutrition, Chung Shan Medical University, Taiwan, R.O.C*
[3]*Institute of medicine, Chung Shan Medical University, Taiwan, R.O.C*
[4]*Department of surgery, Chung Shan Medical University Hospital, Taiwan, R.O.C*

(received November 30, 2019)

ABSTRACT *Background.* Meeting the nutritional needs of preterm infants represents a huge challenge. According to previous studies, higher intakes of protein and energy in the first week of life are linked to improved neurodevelopmental outcomes. However, whether these global guidelines can be successfully implemented at the local level requires further study. *Methods*. This is a retrospective cohort study, for the period from Oct 2016 through Nov 2017. All participating subjects reached 75% of the nutritional targets within one week of birth as the standard of care. But given the clinical condition constraints, they almost inadequate nutrition in the first weeks of life. We conducted a trial involving 122 preterm divided into four groups to determine the most appropriate nutritional strategy: HCHP (more calories and higher protein intake); LCLP (fewer calories and lower protein intake); HCLP (more calories and lower protein intake) and LCHP (higher protein intake and fewer calories). *Results*. Higher energy intake in the first week after birth was related to significant decreases in the average duration of hospitalization ($p<0.05$) and significant weight gain ($p<0.05$). Adequate caloric intake is more important than high protein intake. Furthermore, early aggressive nutritional strategy may help to decreases in length of ICU stay. (HCLP versus LCLP group, odds ratio 0.022 [95% CI 0.003-0.189]). *Conclusion*. Based on the results of this study, we determined the optimal nutritional support for preterm infants. Protein is an important factor in developmental outcomes and can be used most efficiently when associated with adequate caloric intake.
Keywords: Nutritional Requirements; Nutritional Support; Nutrition Therapy; Premature Infant.

INTRODUCTION

Early aggressive nutrition is essential for preterm infant growth and immunity. In preterm infants, poor nutrition is associated with poor head growth and persistent small head size results in poor psychomotor and mental skills and high rates of cerebral palsy and autism (1). Preterm infants are at a higher risk of growth and developmental disabilities compared to their full-term counterparts. Early administration of optimal nutrition to preterm infants lowers the risk of adverse health outcomes and improves cognition in adulthood (2). Recommendations for the nutritional management of preterm infants by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the American Academy of Pediatrics (AAP) and the Taiwan Society of Neonatology (TSN) Committee on Nutrition include higher intakes of protein and energy in the first week of life, which have been linked to improved neurodevelopmental outcomes (3). Amino acids and proteins are key factors for growth. Current recommendations are designed to provide nutrients to approximate the rate of growth and composition of weight gain for a normal fetus of the same postmenstrual age (4). The prevailing nutritional practices for preterm infants include energy requirements of 110~130 kcal/kg/day and protein intake of 3.5~4.5 g/kg/day (5). Various disciplines have contributed specialized expertise to the identification of potentially better practices (6). Although the Committee on Nutrition of ESPGHAN announced appropriate recommendations in 2007, it was considered necessary to review them (8). The updated guidelines are consistent with, but not completely identical to, the major recommendations prior to 2010. However, whether these global guidelines can be successfully implemented on a local level requires further study. Differences in medical care and social factors among countries may limit the generalizability of global guidelines (13). The aim of this study is to review and discuss the manner in which the postnatal growth of preterm infants is monitored in neonatal intensive care units (NICUs) and to investigate whether growth and clinical outcomes are associated with the adequacy of postnatal nutrient intake.

METHODS

This was a retrospective cohort study, conducted for the period from Oct 2016 to the end of Nov 2017, on preterms admitted to the NICU at local medical

*To whom correspondence should be addressed: cshc188@csh.org.tw, mygodway@gmail.com

center and who met the criteria for inclusion. Those who died within 7 days were excluded, of which there were two. Therefore, mortality rate was not considered an indicator. In addition, infants who remained in the hospital at the end of the study period were excluded (Figure 1). All participating subjects reached 75% of the nutritional targets within one week of birth as the standard of care. Due to clinical constraints, almost all received inadequate nutrition in the first weeks of life. The nutritional protocol was to start parenteral nutrition on day 1 and first enteral feed as soon as possible after birth. Continued provision of appropriate nutrition (fortified human milk or premature formula) is important throughout the hospitalization period. Subjects were assigned to one of four groups based on number of calories and amount of protein ingested daily during the first seven days of admission to NICU: high calorie intake group (HCLP) which received more than 75% of the energy needs (90 kcal/kg/day); high protein intake group (LCHP) which received at least 75% of the protein requirement (3 g/kg/day); high calorie and protein intake group (HCHP) which received at least 90 kcal/kg/day with 3 g/kg/day of protein and low calorie and protein intake group (LCLP group) which received less than 90 kcal/kg/day and less than 3 g/kg/day of protein. Perinatal and neonatal data were retrieved from medical records (Figure 1). Weight gain and blood biochemical values were collected after two weeks of nutritional support. Complication rates and lengths of hospital stay were also analyzed. Finally, we explored the impact of nutritional support on the clinical outcomes of preterm infants. This study was conducted according to the guidelines of the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board of Chung Shan Medical University Hospital (CSMUH IRB No: CS-18256).

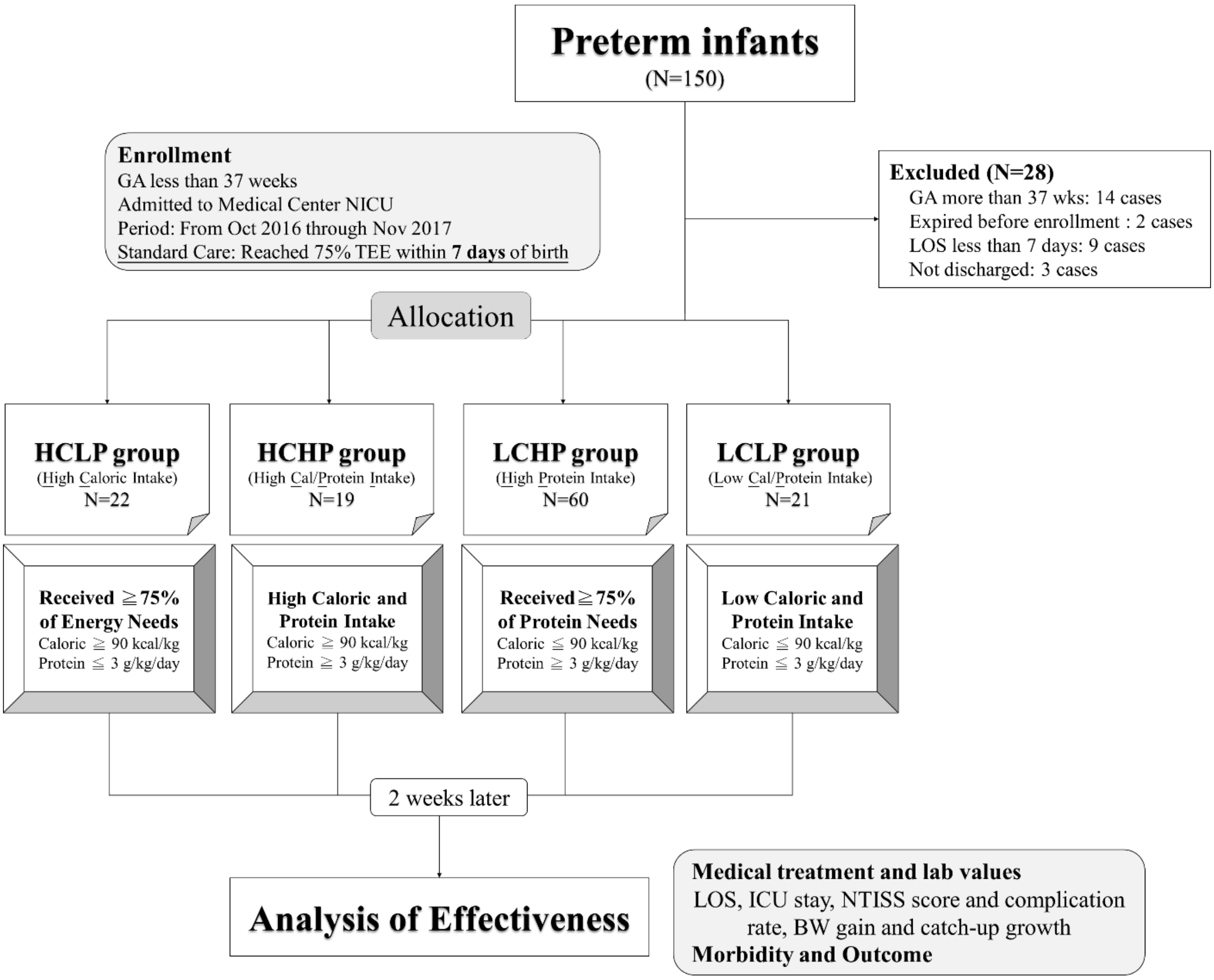

Figure 1. Enrollment flow chart and study protocol

Characteristics

The Neonatal Therapeutic Intervention Scoring System (NTISS) was used to indicate disease severity in neonates requiring intensive care (14). NTISS scores were calculated on the first day and at 7-14 days after admission. The predictive power for clinical outcomes included the average duration of hospitalization (length of stay, LOS) and number of days in ICU. Furthermore, preterm infants can suffer from morbidities such as patent ductus arteriosus

(PDA), respiratory distress syndrome (RDS) above grade II, retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), metabolic bone disease, hypoxic ischemic encephalopathy (HIE), congenital intestinal malrotation and spontaneous intestinal perforation (SIP) (15). Neonatal morbidities were diagnosed by pediatric specialist and retrieved from medical records. Complications in this study were defined as the combination of more than two of these morbidities. Preterm infants received surgical intervention including PDA ligation, external ventricular drainage (EVD)/ventriculoperitoneal shunt (V-P shunt) procedure, photoligulation, inguinal hernia repair and enterostomy.

Statistical Analysis

Continuous variables were summarized as medians with interquartile ranges (IQRs) and reported as 25th-75th percentiles. Categorical variable comparisons were performed with Pearson chi-square test or Fisher's exact test. To determine whether the effect of the intervention on the end points was influenced by baseline risk factors, p values for interactions were calculated with one-way ANOVA and post hoc analysis with Scheffé test with threshold for significance of interaction set at $p<0.05$. For continuous parameters in non-normal distribution, Kruskal-Wallis H test was used when the assumptions of one-way ANOVA were not met. We further performed Mann-Whitney U test to compare the differences between two groups. As the group with the highest intakes was comprised of infants of the highest gestational age (GA) and birth body weight (BBW), confounders were GA and BBW. Following initial analysis, logistic regression analysis was performed for variables without statistically significant difference. Furthermore, we adjusted for the confounders GA and BBW during the nutritional treatment period and clinical outcome. Logistic regression was applied to the baseline characteristics and clinical outcome. All analyses were performed using PASW Statistics 18, version 18.0.0 (formerly SPSS Statistics).

RESULTS

A total of 122 preterm infants of less than 37 weeks' gestational age were eligible for this study. The basic characteristics of these infants are shown in Table 1. Infants in the LCLP group had lower GA, lower BBW, and lower Apgar scores at 1 minute and 5 minutes when compared with infants in the HCLP group (all $p< 0.05$). On post hoc analysis with Scheffé test, there were statistically significant differences in GA between the HCLP and LCHP groups and the HCLP and LCLP groups. Similarly, there were statistically significant differences in BBW and Apgar score at 1 minute. We performed logistic regression analysis with the significant variables BBW and GA. After adjusting for significant factors, there were no significant differences between groups at baseline as presented in Table 1.

In addition, there were no associations with laboratory data after 2 weeks of nutritional support, such as hemoglobin, white blood cell count and platelet count. Similar results were found for electrolyte balance (data not shown). Median C-reactive protein levels were within normal range with decreased inflammation in all groups at 2 weeks. Unfortunately, information about nutritional status and possible indexes to be applied to organ function maturity was limited. Due to insufficient data on serum albumin, alkaline phosphatase, bilirubin level, γ-glutamyltransferase and creatine kinase, these could not be analyzed.

Adequate nutritional support was associated with improved growth and clinical outcomes and less extrauterine growth retardation (EUGR) (Table 2). High energy with low protein intake during the first week after birth was associated with significant decreases in the average duration of hospitalization and length of ICU stay (HCLP versus LCLP group both $p<0.01$, respectively). Higher energy with higher protein intake was only associated with significant decreases in length of ICU stay (Figure 3), indicating an excellent predictive marker for clinical outcome. Additionally, HCLP group showed significantly improved weight gain at 2 weeks when compared with LCHP group and LCLP group ($p<0.05$, Table 2).

Although administration of optimal nutrition resulted in lower risk of more than two preterm complications and surgical intervention for morbidities (Adjusted $p>0.05$), there were significant differences in ICU stay between HCLP versus LCLP groups and HCHP versus LCLP groups (Figure 3). For HCLP versus LCLP group, odds ratio was 0.022 [95% CI 0.003-0.189], $p=0.001$ and for HCHP versus LCLP group, odds ratio was 0.044 [95% CI 0.006-0.33], $p=0.002$. Moreover, LCLP group showed significantly increased LOS (HCLP versus LCLP group, odds ratio 0.022 [95% CI 0.002-0.267], $p=0.003$).

DISCUSSION

Providing more than 75% of the nutritional needs to preterm infants within 7 days of birth can improve clinical prognosis. Protein administration is more effective when number of calories is adequate. Although survival of premature infants has improved, there is a continuing need to develop and implement strategies for reducing the potentially lethal complications of premature birth (16). Early aggressive nutritional enteral and parenteral support may help to improve growth and developmental outcomes in preterm newborn low birth weight (LBW) infants (12). In preterm infants, poor postnatal growth is associated with adverse neurocognitive outcomes. Conversely, rapid postnatal growth is considered a risk factor for future development of metabolic diseases (17). Therefore, sufficient nutritional support and optimal nutritional delivery are essential. Protein-energy balance studies of preterm infants have provided data to guide recommendations for protein-energy intakes for specific short-term goals (18). Nevertheless, from the results of our study on the relevance of energy it is particularly important to promote protein balance at lower energy intakes, as amino acids are increasingly used for oxidative metabolism when non-protein energy is limited. Regardless of protein intake, however, nutritional balance requires increased energy intake. A reasonable explanation for no associations with laboratory data in this study is that the biochemical values of premature infants are monitored daily and corrected immediately.

Table 1. Baseline characteristics of patients at enrollment

Characteristics[α]		HCLP 90kcal	HCHP 90kcal+3g	LCHP 3g	LCLP None	p value[β]	Adjusted p[γ]
Number		22 (18.1)	19 (15.6)	60 (49.2)	21 (17.2)	-	-
Male		12 (54.5)	7 (36.8)	35 (58.3)	14 (66.7)	0.268	-
GA (wk)		35.86 [ab] (34.1-36.3)	35 [c] (33.9-35.4)	33.07 [a] (30.3-34.3)	33.86 [bc] (27.4-34.9)	<0.01	0.119
BL (cm)		45.3 (42-48.3)	45.5 (42-47.5)	42 (39-46)	42 (35.3-47.5)	0.027	0.121
BBW (kg)		2.300 [de] (1.863-2.550)	2.020 (1.730-2.269)	1.756 [d] (1.301-2.066)	1.758 [e] (0.975-2.252)	<0.05	0.66
Apgar Score	1 min	8 (7-8.25) [fg]	8 (7-8) [h]	7 (6-8) [f]	6 (5-7.5) [gh]	<0.01	0.138
	5 min	9 (9-9) [i]	9 (9-9)	9 (7-9)	8 (7-9) [i]	<0.01	0.439

[α] Continuous variables are presented as median (IQR) and categorical variables as n (%)
[β] The p values are from Pearson chi-square test for categorical variables and from one way ANOVA or Kruskal-Wallis test for continuous variables.
[γ]Adjusted p values are from binary logistic regression analysis after adjusting for significant factors. (Confounding variables: BBW, GA)
[*] The same English symbols indicate significant difference

Table 2. Clinical outcomes of each group

Intervention Period	Prognosis assessment					
	Nutritional therapy groups				p-value	Adjusted p
	HCLP	HCHP	LCHP	LCLP		
Actual Calories (kcal/kg BW)	104.6 (99-113.5)	101.5 (92.5-117.8)	74.2 (66.2-79)	57 (51.7-71.7)	<0.01	-
Actual Protein (g/kg BW)	2.56 (2.29-2.67)	3.67 (3.38-4.09)	3.74 (3.46-4.11)	2.83 (2.52-2.95)	<0.01	-
LOS (days)	14.5 [a] (12.8-21.5)	28 (14-37)	39.5 (27.3-74.3)	61 [a] (30-97.5)	**<0.01**	**0.025**
ICU stay (days)	7.5 [b] (5-12)	12 [c] (7-21)	23 (15.3-51.5)	46 [bc] (17-71.5)	**<0.01**	**<0.01**
BW gain (gm)	290 (171-357)	188.5 (65.8-258.3)	91.5 (36.8-155.8)	101 (36-179.5)	**<0.01**	**0.015**
NTISS score (after 7~14 days)	8 (5-10.5)	9 (7-9)	9.5 (7-15)	10 (7-21.3)	0.087	-
Complications	3 (14)	5 (26)	28 (47)	14 (67)	**<0.01**	0.287
Surgery	0 (0)	2 (11)	13 (22)	10 (48)	**<0.01**	0.437

[*] Continuous variables are presented as median (IQR) and categorical variables as n (%)
[*] The same English symbols indicate significant difference
[*]Adjusted p values are from binary logistic regression analysis after adjusting for significant factors. (Confounding variables: BBW, GA)

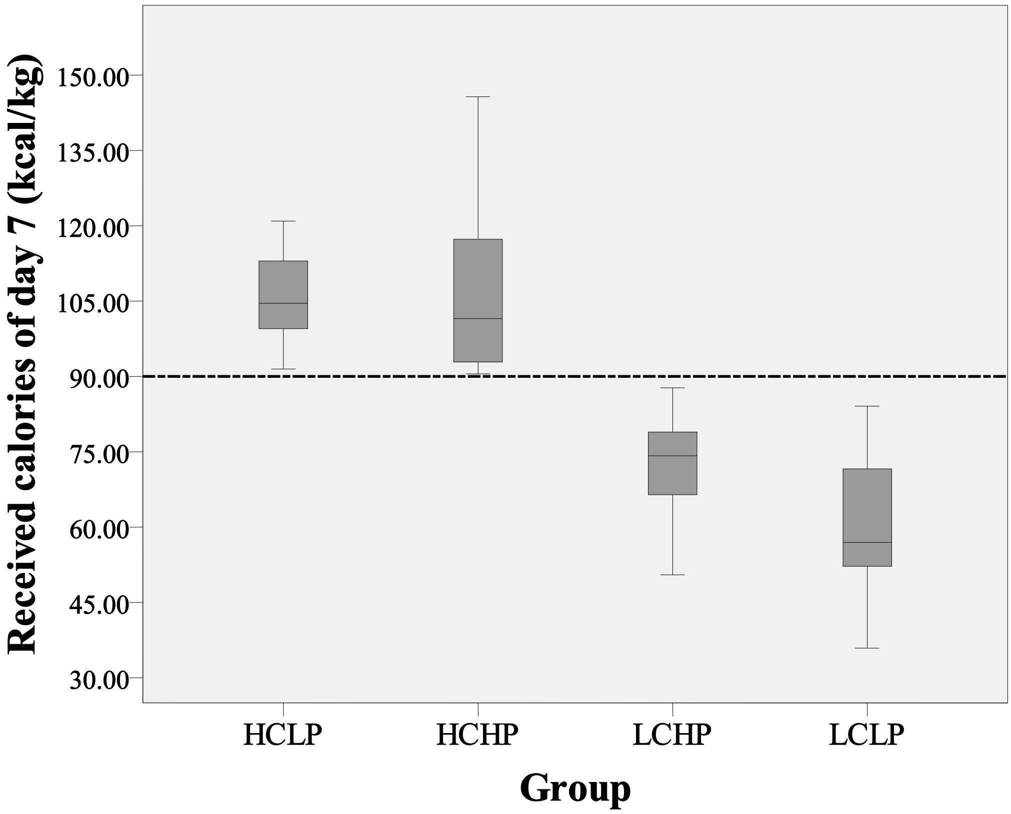

Figure 2a. Actual caloric intakes of each group. There were significant increases in HCLP and HCHP group when compared with LCHP and LCLP groups.

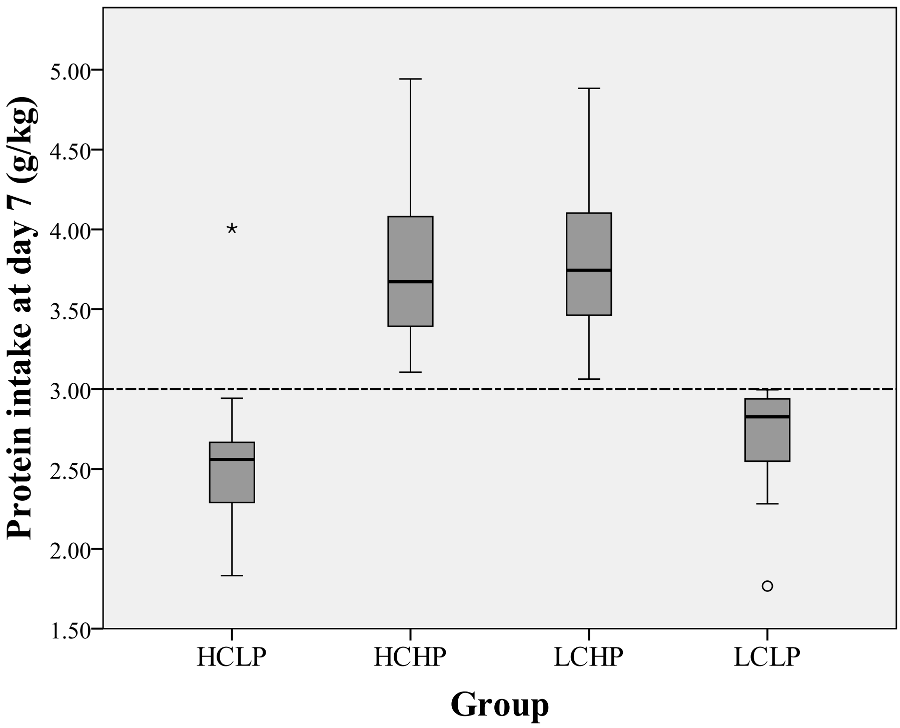

Figure 2b. Actual protein intakes of each group. There were significant increases in HCHP and LCHP group when compared with HCLP and LCLP groups.

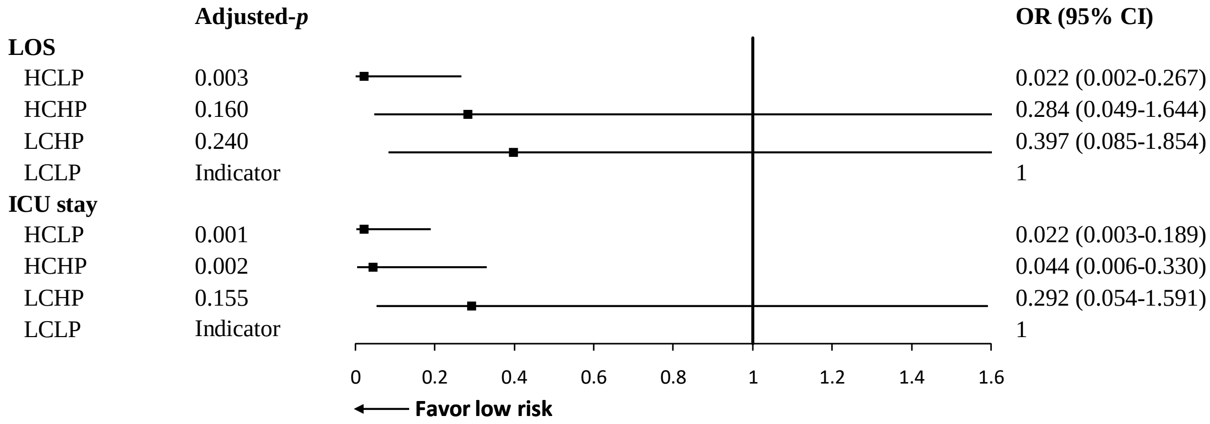

Figure 3. The relevance of this experiment. HCLP group showed significant improvement in odds ratios of LOS and ICU stays when compared with LCLP groups.

Previous studies have suggested that increasing energy intake in the face of static protein intake should be avoided in the first week of life (19). As there is often a gap between global guidelines and the results of clinical practice, NICUs should develop their own methods of nutritional support for preterm infants suited to local conditions and actual patient needs. Our study of a local practice revealed the optimal nutritional support strategy for preterm infants. Greater energy and lipid intake predict increased total brain and basal nuclei volumes over the course of neonatal care to term-equivalent age (20). Evidence linking postnatal weight gain to later adiposity and other cardiovascular disease risk factors in preterm infants is limited (17). In our study, more than 90 kcal/kg/day with or without 3 g/kg/day protein was associated with sufficient weight gain (> 150 g/week). Consistent with the findings of a previous study, emphasis should be on providing optimal energy and protein during the first week following birth (21).

A previous study proposed that infants weighing ≥1250 g be fed three times hourly and those weighing <1250 g be fed two times hourly (22). The Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (ASPEN) recommend that enteral feeding protocols be designed and implemented to increase the overall percentage of goal calories provided. For instance, volume-based feeding protocol or top-down multi-strategy protocol should be considered (23). Protocols in which 24-hour or daily volumes are targeted instead of hourly rates have been shown to be successful in increasing the overall percentage of goal energy provided. Consequently, daily volumes may be applied to preterm feeding protocol for catch-up growth and early aggressive nutritional support.

The major limitation of this study is that enteral nutrition and parenteral nutrition were not calculated separately, although each has different requirements. We started parenteral nutrition on day 1 and first enteral feed as soon as possible after birth. However, their effects could not be differentiated. Correspondingly, determining simple causes of clinical outcome when multiple causes may play a role can be difficult and subjective. We used regression statistics to minimize bias in determining variables, but with uncertain validity. From observational studies there are consistent positive associations between nutritional support and clinical outcomes. However, there is limited evidence from intervention studies. Given the above constraints, further studies are needed to clarify the effects of early nutritional intervention on preterm infants.

ACKNOWLEDGEMENT

We would like to thank the anonymous reviewers and the editor for their comments. In addition, we would like to acknowledge all participating investigators and the directors of the nutrition and pediatric departments of hospital who served as scientific advisors. A research assistant from university school collected data and attending physicians in the NICU of hospital provided care to the study subjects.

REFERENCES

1) Hayes B, Lee K. Head size and growth in the very preterm infant: a literature review. Res Reports Neonatol 5:1-7. 2015.
2) Kumar RK, Singhal A, Vaidya U, Banerjee S, Anwar F, Rao S. Optimizing Nutrition in Preterm Low Birth Weight Infants-Consensus Summary. Front Nutr 4:20. 2017.
3) Koletzko B, Poindexter B, Uauy R, editors. Nutritional Care of Preterm Infants: Scientific Basis and Practical Guidelines. 2014. Karger, Basel.
4) Riley LE, Stark AR, Kilpatrick SJ. Guidelines for Perinatal Care, 7th ed. 2012. American Academy of Pediatrics and American College of Obstetricians and Gynecologists, Elk Grove Village, IL.
5) Senterre T. Practice of enteral nutrition in very low birth weight and extremely low birth weight infants. World Rev Nutr Diet 110:201-14. 2014.
6) Wight N, Kim J, Rhine W, Mayer O, Morris M, Sey R, eds. Nutritional Support of the Very Low Birth Weight (VLBW) Infant: A Quality

Improvement Toolkit. 4th ed, 2018. California Perinatal Quality Care Collaborative, Stanford CA.

7) Ziegler EE. Protein requirements of very low birth weight infants. J Pediatr Gastroenterol Nutr 45(Suppl 3):S170-174. 2007.

8) Agostoni C, Buonocore G, Carnielli VP, Curtis MD, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: Commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 50:85-91. 2010.

9) Edmond K, Bahl R, eds. Optimal feeding of low-birth-weight infants: technical review. 2006. World Health Organization, Geneva Switzerland.

10) Su BH. Optimizing Nutrition in Preterm Infants. Pediatr Neonatol 55(1):5-13. 2014.

11) Alto C, Nash M, Markell A, Niemeyer J, Mitch A, Stawarz M, eds. Nutrition Practice Care Guidelines for Preterm Infants in the Community, 5th ed. 2016. Oregon Pediatric Nutrition Practice Group, Seattle.

12) Ehrenkranz RA. Early, aggressive nutritional management for very low birth weight infants: what is the evidence? Semin Perinatol 31(2):48-55. 2007.

13) Kim GH. Gaps between Global Guidelines and Local Practices in CKD-MBD. Electrolyte Blood Press12(2):35-40. 2014.

14) Wu PL, Lee WT, Lee PL, Chen HL. Predictive power of serial neonatal therapeutic intervention scoring system scores for short-term mortality in very-low-birth-weight infants. Pediatr Neonatol. 56(2):108-113. 2015.

15) Su YY, Wang SH, Chou HC, Chen CY, Hsieh WS, Tsao PN, et al. Morbidity and mortality of very low birth weight infants in Taiwan-Changes in 15 years: A population based study. J Formos Med Assoc 115(12):1039-1045. 2016.

16) Patel RM, Kandefer S, Walsh MC, Bell E, Carlo WA, Laptook AR, et al. Causes and timing of death in extremely premature infants from 2000 through 2011. N Engl J Med 372(4):331–340. 2015.

17) Ong KK, Kennedy K, Castañeda-Gutiérrez E, Forsyth S, Godfrey KM, Koletzko B, et al. Postnatal growth in preterm infants and later health outcomes: a systematic review. Acta Paediatr 104(10):974-986. 2015.

18) Bhatia J, Mena P, Denne S, García C. Evaluation of Adequacy of Protein and Energy. J Pediatr 162(3):S31-36. 2013.

19) Christmann V, Roeleveld N, Visser R, Janssen AJWM, Reuser JJCM, Goudoever JBV et al. The early postnatal nutritional intake of preterm infants affected neurodevelopmental outcomes differently in boys and girls at 24 months. Acta Paediatr 106(2):242-249. 2017.

20) Schneider J, Fischer Fumeaux CJ, Duerden EG, Guo T, Foong J, Graz MB, et al. Nutrient Intake in the First Two Weeks of Life and Brain Growth in Preterm Neonates. Pediatrics 141(3):e20172169. 2018.

21) Stephens BE, Walden RV, Gargus RA, Tucker R, McKinley L, Mance M, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123(5):1337-1343. 2009.

22) Dutta S, Singh B, Chessell L, Wilson J, Janes M, McDonald K, et al. Guidelines for feeding very low birth weight infants. Nutrients 7(1):423-442. 2015.

23) McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, et al. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient. J Parenter Enter Nutr 40(2):159-211. 2016.

ORIGINAL

Validation of a Pediatric Nutrition Screening Tool in Hospital Outpatients of Myanmar

Lin Ei Phyu[1], Wantanee Kriengsinyos[1*], Nipa Rojroongwasinkul[1], Nalinee Chongviriyaphan[1], Tippawan Pongcharoen[1]

[1]*Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand*

(received Jan 20, 2020)

ABSTRACT *Background:* Nutrition screening is important in identifying children at risk of developing malnutrition. No pediatric nutrition screening tool is previously applied or validated in Myanmar. Objective: This study aimed to validate Screening of Risk for Nutritional Status and Growth (STRONGkids) tool and to analyze the association of nutrition status with the clinical characteristics of Myanmar pediatric outpatients. *Method:* The STRONGkids screening score was calculated and the nutrition risk from the tool was compared with the WHO growth standards determined by weight and height related z-scores. The nutrition status of the participants and its association with clinical factors were also investigated. *Results:* A total of 120 children (60 boys, 50%), aged between 1 and 12-year-old, were included. The screening tool identified 58.3% of children as nutritionally-at-risk. It had 90.9% sensitivity and 45% specificity to detect thinness, and 81% sensitivity and 46.5% specificity for stunting. The nutrition risk from the screening was also significantly associated with the weight, height, and BMI-related WHO z-scores ($p < 0.05$). Overall, 26.6% of our study children had thinness and/or stunting, and > 5-year old children had significantly reduced weight status compared to the younger age group. *Conclusion:* This study suggested that the STRONGkids screening tool is a sensitive and valid tool that can be used for early detection of malnutrition in Myanmar pediatric outpatients. The effectiveness of nutrition intervention following screening should be further investigated.

Keywords: Malnutrition; Pediatric; Nutrition Screening Tool; Myanmar; Anthropometry

INTRODUCTION

Childhood malnutrition is considered as a global health concern since it is associated with poor growth and development, as well as reduced educational outcomes of children and can have negative impacts on their adulthood (1). The 2018 global malnutrition report estimated that the prevalence of under-five malnutrition in the form of wasting was around 49 million, and stunting was around 149 million (2). Undernutrition is not only a consequence of prolonged starvation or food insecurity but also diseases, injuries or illness. Children with chronic diseases and hospitalized children have a greater risk of malnutrition since they have increased energy demand from the diseases, and reduced nutrient intakes and absorption from underlying conditions, medications and, or, inadequate nutritional support during the treatment (3). On the other hand, malnourished children have an increased risk of infections, poor healing and disease-associated complications, which can increase their morbidity and mortality (3, 4). Therefore, early identification of nutritional risk in children is essential in order to prevent from severe malnutrition and its complications (5, 6). International organizations such as the American Society for Parenteral and Enteral Nutrition (ASPEN) and the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), thus, recommend the early detection of malnutrition risk by screening (7). Several nutrition screening and assessment tools have recently been developed, but the agreement regarding the best screening tool has not reached yet (3, 8). Although nutritional screening tools are developed with pre-specified nutritional intervention plan, the successful implementation of this plan during hospitalization is limited for some patients due to decreased length of hospital stays. In contrast, if a screening tool can be applicable to the outpatient setting, followed by detailed nutritional assessment, the optimal benefit from timely nutrition intervention can be achieved. Almost all of the previous screening tools were developed for hospitalized children and the applicability of these tools in outpatient population is still needed to be investigated.

In the outpatient setting of Myanmar hospitals, although physicians could recognize the children who are already malnourished, the lack of a validated screening tool makes it difficult to diagnose the children who are at risk of malnutrition. In addition, a detailed nutritional assessment cannot be performed in every pediatric outpatient since it is a time-consuming process which required skills and knowledge in

*To whom correspondence should be addressed:
Wantanee Kriengsinyos
<wantanee.krieng@mahidol.ac.th>

nutrition. Therefore, there is a probability of missing children who were at-risk to be malnourished and did not receive timely nutritional treatment. The application of nutritional screening tool in outpatient clinic can detect the children at risk at an early point, and can prevent from consequences of malnutrition. For the practical application in outpatient clinical practice, a malnutrition screening tool should be quick, simple, reliable and easy to understand. Therefore, our study aimed to validate the Screening of Risk for Nutritional Status and Growth (STRONGkids) tool which has been reported as an easy-to-use and rapid screening tool (6, 9), and furthermore, to evaluate the factors associated with nutrition status in Myanmar pediatric outpatients.

METHODS

This cross-sectional study was conducted during February to April 2019 in pediatric outpatient department of Parami General Hospital, which is a private medical center located in Yangon, and providing health care services especially for the children. The study was approved by Mahidol University Central Institutional Review Board (MU-CIRB 2019/029.1102).

Validation of nutrition screening tool

In order to validate a screening tool, the nutrition status based on WHO anthropometric indicators: weight-for-age (WFA), weight-for-height (WFH), height-for-age (HFA) and BMI- for-age were chosen as a trusted criterion standard. Malnutrition as defined by World Health Organization is the presence of either wasting (WFH z-score <-2SD or BMI-for-age z-score < -2 SD), stunting (HFA z-score < -2 SD) or underweight (WFA z-score < -2 SD) (10). The patients with each of these anthropometric z-score of < -2SD were considered as malnourished, and ≥-2 SD were considered as well-nourished.

Subject selection and data collection

The pediatric outpatients who aged 1 years or older and whose parents agreed to participated in the study were included in the study. Critically ill children, and the children with inability to perform anthropometric measurements were excluded. All of the subjects were recruited by convenient sampling, and data collection was initiated after getting the informed consent from the parents. The application of screening tool and anthropometric assessment were performed on the same day by two different researchers.

Anthropometry

The weight measurement was done with the children on light clothes and recorded to the nearest 10 g, on the electronic scale accurate to at least 100g (11). Height was recorded to the nearest 0.1 cm, and supine length was measured for children under 2years of age. Mid-upper Arm Circumference (MUAC) was measured in children younger than 5 years old, by using the measuring tape in the left upper arm of the child, at the mid-point between olecranon process and acromion. The anthropometric measurements were classified as z-scores corresponding to age and sex according to WHO growth reference, and these were calculated by using the WHO Anthro version 3.2.2 and WHO Anthro Plus software (12).

Nutrition Screening

The caregivers or older children in the study were interviewed with the questions in the STRONGkids nutrition screening tool (9) which includes 1) the presence of illness with nutrition risk or plan for surgery, 2) physical appearance by subjective clinical assessment, 3) indicators of reduced intake such as gastrointestinal symptoms, pain, reduced food intake, nutritional intervention and presence of pain, and 4) weight history. The scoring of 1 point was given to any positive answer the questions except the presence of underlying disease and given with the weighted score of 2 points. Therefore, the total score for all positive response is 5 points and the children were categorized into three groups; high risk (total score ≥ 4), moderate risk (total score = 1 to 3), and low risk (total score = 0).

Dietary evaluation

A single 24-hour dietary recall of the children during their illness was taken from the caregivers or older children to estimate the approximate energy intake. The energy intake of the children was compared with the age-specific recommended dietary allowance per day for Southeast Asia (13), in order to decide whether they had an adequate caloric intake (≥ 75% of RDA) or inadequate caloric intake (< 75% of RDA) during illness (14).

Statistical analysis

Descriptive statistics were used for presenting patient characteristics, anthropometric data and other categorical variables. Based on the weight and height related z-scores and the cut-off point of -2 SD for malnutrition, the sensitivity, specificity, positive predictive value and negative predictive value of the nutrition screening tool was determined. In the contingency table, medium and high-risk categories from the tool were combined as "at-risk" category, and the low-risk was considered as "not-at risk" category in order to calculate these diagnostic values of the tool. The chi-square method, or exact Fisher's test when appropriate, was applied to determine the presence of a significant association between dichotomous variables such as nutritional risk (at-risk and not-at-risk), age (< 5 years and ≥ 5 years), gender (male, female) and caloric intake (adequate, inadequate) and disease status (acute and chronic) with the nutritional status by WHO z-scores (well-nourished and malnourished). The agreement of the screening tool with anthropometry was decided by calculating Cohen κ statistics, with 95% confidence intervals, and interpreted using value scores by Landis and Koch (15). The sample size for the validation was calculated by expecting the Cohen's kappa coefficient κ value would be at least 0.4, which was considered to be appropriate based on previous report (15). With the significance level of 5%, power of 90% with two tails, the minimum sample of 62 is required for kappa at 2×2 category, according to sample size calculation guideline using Cohen's kappa value by Bujang et. al (16). However, in order to avoid the possibility of incomplete data, we accounted a doubled sample size (16). All the statistical calculations were done by using computer software, IBM SPSS Statistics version 22.0 (IBM Corp. Armonk, NY, USA). The p value <0.05 was considered statistically significant.

RESULTS

Among the families approached in the outpatient department during the study period, there were 120 eligible pediatric outpatients (50% males) who completed both anthropometric assessment and nutrition screening tool. The median age of the patients was 3.3 years (range between 1 to 10 years). There were 85 children (70.8%) who aged below 5 years old, and 35 children (29.2%) aged 5 years or older. Majority of the children (84.2%) were presented with acute illness including seasonal flu and viral or bacterial infections of respiratory tract, urinary tract or skin, gastroenteritis and others. Only 15.8% of the patients had chronic disease conditions such as congenital heart disease, tuberculosis and chronic respiratory diseases. According to the 24-hr dietary recall of the children, we found that there were 26 children who had inadequate caloric intake (< 75% of the recommended daily allowance) during their illness (Table 1). Moreover, it was also observed that more than half (55%) of this outpatient population were currently taking multivitamin supplements.

Prevalence of undernutrition among study participants

Among the 120 patients studied, the WFH z-score was determined in 86 children who were 5 years old or younger. There were 6 children who had wasting (WFH z-score < -2 SD) with one of them being severely wasted (WFH z-score <-3 SD). The same age group was examined for MUAC z-score and no children in this group had their MUAC z-score less than or equal to -2 SD. The WFA z-scores was calculated in children younger than 10 years (n=118) and there were 14 children who were underweighted and the remaining 88.1% had normal weight. BMI-for- age z-score was also calculated for children of all age groups and 9.1% of them (n=11) had thinness. It was also found that 21 children in our study had stunting (HFA z-score < -2 SD) or chronic malnutrition. Overall, acute malnutrition was found in 9.1% and chronic malnutrition was diagnosed in 17.5 % of our sample (Table 2).

Table 1. General characteristics of study children

Characteristics		No. (n=120)	%
Age (yr)	<2	28	23.3
	≥2 to <5	57	47.5
	≥5	35	29.2
Gender	Male	60	50
	Female	60	50
Disease	Acute	101	84.2
	Chronic	19	15.8
Diagnosis	Infection/fever	44	36.7
	Respiratory	43	35.8
	Gastrointestinal	21	17.5
	Cardiac	1	0.8
	Others	11	9.2
Caloric intake*	Adequate	94	78.3
	Inadequate (<75% of RDA)	26	21.7

**Caloric intake calculated from 24-hr food recall (intake during illness)*
RDA, recommended daily allowance

Table 2. Anthropometric characteristics of the study children

Anthropometric indicator	Number of children, n (%)		
	≥-2SD	< -2SD to -3SD	<-3SD
WFH z-score(n=86)	80(93)	5(5.8)	1(1.2)
HFA z-score(n=120)	99(82.5)	19(15.8)	2(1.7)
WFA z-score(n=118)	104(88.1)	10(8.5)	4(3.4)
BMI for age z-score(n=120)	109(90.8)	7(5.8)	4(3.3)
MUAC z-score (n=86)	86(100)	0(0.0)	0(0.0)

WFH, weight-for-height; HFA, height-for-age; WFA, weight-for-age; BMI, body mass index; MUAC, mid-upper arm circumference

Validity of STRONGkid nutrition screening tool in hospital outpatient setting

According to nutrition screening by STRONGkids tool, 58.3% of our study population (n=70) had moderate nutrition risk and the remaining children had low or no risk of malnutrition. None of the participants from our study had high risk of malnutrition. When the nutrition risk was compared to WHO anthropometric indicators, it has 100% sensitivity and 47.5% specificity in identifying wasting, and 81% sensitivity ad 46.5% specificity in identifying stunting. Overall, the tool has an excellent sensitivity (>90%) except the comparison with HFA z-score (81%), and fair specificity (>45%) in detecting malnutrition. When compared to WHO standards of weight for height, weight for age, BMI-for-age and height for age, it was found that the screening questionnaire had significant association with wasting, underweight and stunting with p-value < 0.05. However, the kappa agreement between anthropometry and nutrition risk was still weak (□= 0.105 to 0.143) (Table 3).

Characteristic of 120 pediatric outpatients in relation to their nutritional status

Among the under-five years old children (n=85), 7.1% had wasting, 7.1% had underweight and 20% had stunting according to WHO standards. In the children who aged 5 years or older, 24.2% had underweight, 20% had thinness and 11.4% had stunting. Between these two age groups, the WFH and HFA z-scores were not significantly different. However, the older age group had significantly lower WFA and BMI-for-age z-scores ($p < 0.05$) than the younger ones. According to our data, different forms of acute malnutrition such as wasting, underweight and thinness were more common in boys compared to girls, 7.7%, 15% and 11.7% respectively. The percentage of chronic malnutrition or stunting in girls was more than boys (18.3% compared to 16.7%). However, there was no statistically significant difference in characteristics of the patients such as sex, and acute or chronic disease status in both well-nourished and malnourished groups, except the inadequate caloric intake calculated from 24-hour dietary recall, which had a statistical association with stunting (p=0.02) (Table 4).

Table 3. Cross-classification of nutrition risk from screening and WHO anthropometric standards

Nutrition risk	WFH z-score (n=86)		WFA z-score (n=118)		BMI-for-age z-score (n=120)		HFA z-score(n=120)	
	<-2 SD	≥-2SD	<-2 SD	≥-2SD	<-2 SD	≥-2SD	<-2 SD	≥-2SD
Risk (n)	6	42	13	57	10	60	17	53
No risk (n)	0	38	1	47	1	49	4	46
p-value	0.032[b]		0.007[a]		0.025[b]		0.02[a]	
Kappa	0.112		0.139		0.105		0.143	
Sensitivity	100		92.9		90.9		81	
Specificity	47.5		45.2		45		46.5	
PPV	12.5		18.6		14.3		24.3	
NPV	100		97.9		98		92	

[a]chisquare; [b]fisher's exact test

WFH, weight-for-height; WFA, weight-for-age; BMI, body mass index; HFA, height-for-age; PPV, positive predictive value, NPV; negative predictive value

Table 4. Association between clinical characteristics and nutrition status of children

	Wasting		Underweight		Thinness		Stunting	
	n (%)	p	n (%)	p	n (%)	p	n (%)	p
Age								
<5yr (n=85)	6(7.1)	1.00	6(7.1)	0.02*	4(4.7)	0.01*	17(20)	0.30
≥5yr (n=35)	0 (0)		8(24.2)		7(20)		4(11.4)	
Gender								
Male (n=60)	3(7.7)	1.00	9(15)	0.40	7(11.7)	0.53	10(16.7)	1.00
Female (n=60)	3(6.4)		5(8.6)		4(6.7)		11(18.3)	
Disease								
Acute (n=101)	6(8)	1.00	12(12)	1.00	10(9.9)	1.00	19(18.8)	0.52
Chronic(n=19)	0(0)		2(11.1)		1(5.3)		2(10.5)	
Caloric intake								
Adequate (n=94)	4(6.0)	0.61	8(8.7)	0.08	9(9.6)	1.00	12(12.8)	0.02*
Inadequate (n=26)	2(10.5)		6(23.1)		2(7.7)		9(34.6)	

p-value for association between categorical variables were derived from Fisher's exact test

DISCUSSION

In Myanmar, the applicability of nutrition screening tool has never been studied in hospitalized children, or, in children at the outpatient department. To the best of our knowledge, this is the first study to evaluate the efficiency of a nutrition screening tool in detecting risk of malnutrition among children in a hospital outpatient setting of Myanmar.

The STRONGkids nutrition screening tool has been validated and widely used in many developed and developing countries (17, 18). For the validation of this tool in Myanmar population, we chose the anthropometric measurements which have been used globally to assess the malnutrition, as a reference method (6) for comparison. The validation of the screening tool in our study demonstrated a good sensitivity and negative predictive value (> 90%) in detecting different forms of acute malnutrition of the children (Table 3). This value was higher than the validation of STRONGkids tool in Belgium which presented 71.9% sensitivity for identifying acutely malnourished pediatric inpatients (9). Alternatively, when comparing to WFH z-score, our screening tool has slightly lower specificity (47.5%) than the study by Huysentruyt et al which has 49.1% specificity (9). According to ASPEN, the purpose of the screening in a clinical setting is to filter out the children with no risk of undernutrition, and to find the children who may be benefitted from full nutritional assessment and intervention (19). Therefore, the higher false positive rates will only increase the number of patients who undergo full nutritional assessment and, for the purpose of screening, sensitivity is more important than the specificity (9).

Previously, few studies had been conducted to investigate the value of nutritional screening in pediatric outpatients, although most of the tool have applied and validated for hospitalized children in developed and developing countries. The STRONGkids screening tool was studied in special schools by Joosten et al with the aim of finding association between subjective health status and nutrition risk, and it was reported that children with nutrition risk had more difficulties in performing daily activity and increased tendency to have pain (20). Another tool, the Screening Tool for the Assessment of Malnutrition in Pediatrics (STAMP) was also applied in 1-6 years old children at an ambulatory setting, and, in contrast to our screening results which only had weak agreement (□~0.1) with the reference method, the STAMP tool had a moderate agreement with full dietitian assessment (□=0.47(95% CI 0.24-0.70)) (21). This can be explained by the use of different criterion standards for determining nutritional status in validation of screening tool.

In our study population, 7% of children had wasting, 9.1% had thinness and 17.5% were stunted according to WHO growth standards. The overall prevalence of malnutrition in our participants according to anthropometry was 26.6%. This percentage is less than our neighbor country, India by Gupta et al, where the prevalence of malnutrition in OPD children of under five years was reported as 66.4% (22). However, the data in Myanmar comparable to our study results is limited. Among the participants, 70% of children were younger than 5 years old, and wasting and stunting were identified in 7.1% and 20% of them respectively (Table 2). These prevalence rates were in accordance with the recent community malnutrition report by UNICEF/WHO in which 6.6% and 29.4% of under 5 years children in Myanmar had wasting and stunting (2). Although the study was done in a private hospital, there was a recognizable prevalence of malnutrition in children with acute illness. Moreover, we also found that although the weight of the children was measured by the nurses as a routine procedure in outpatient department, the measurement of height and standard procedure of plotting anthropometric measurement on WHO growth charts was not performed routinely. Therefore, the application of a screening tool not only can help in detecting the children with nutrition risk, but also can improve the use of WHO growth chart.

It was also found that there was a statistically significant association ($p<0.05$) between age of the child with the malnutrition status according to WFA and BMI-for-age data (Table 4). Children who were older than 5 years had higher prevalence of underweight and thinness. Similar to our results, the general population prevalence of underweight in 5-19 years old children was also high in Myanmar, which was reported as 33.3% in females and 39.9% in males in 2015(2). No statistical association ($p>0.05$) was found between sex and malnutrition status in our study (Table 4). In contrast, a study performed in Iranian hospitalized children by Gholampour et al demonstrated that the male sex constituted the larger proportion of malnourished group (23).

The results of the current study indicated that 15.8% of the participants had chronic disease and 84.2% had acute illness. It was noticeable that in the group of children with acute fever or other non-specific infections, there was a relatively high rate of chronic malnutrition or stunting (Table 4). Rub et al also reported regarding high malnutrition prevalence which was found in apparently healthy children, and this is comparable with our results (21). The evaluation of 24-hour food intake of the children revealed that reduced caloric intake had statistically significant association with malnutrition risk (Table 4). This indicates that the patients with acute illness had tendency to be malnourished due to reduced oral intake. Moreover, we found that the reduced food intake of patients had statistical association with stunting (Table 4). However, the food intake was taken from a single day during illness, and it cannot be used to explain the high rate of stunting in our population.

There are some limitations in our study. Firstly, this is a single center study with relatively small sample size, and our participants may not represent the whole outpatient population in Myanmar, and the generalization of our results is not possible. The cross-sectional design of our study does not permit evaluating the outcome of nutrition intervention in children with high or moderate risk. Further research which evaluate the effect of presence or absence of nutrition intervention following screening in children at-risk is recommended.

In conclusion, our study is the first one to validate a simple pediatric nutrition screening tool for detecting malnutrition in Myanmar hospital-outpatient setting. The tool was very sensitive and it can detect >90% of the children with poor nutrition status. The specificity of the screening tool was around 50% and there was a significant association of nutrition risk calculated from screening tool with the nutrition status by WHO growth standards. Our results demonstrated a recognizable prevalence of malnutrition among the pediatric outpatients of Myanmar. Furthermore, the present study provided public health information regarding disease and malnutrition status in a private hospital. Together, our results encouraged the use of nutrition screening tool in different hospital settings for early detection of malnutrition and to reduce the prevalence and consequences of malnutrition in Myanmar children.

ACKNOWLEDGEMENT

The author gratefully acknowledged Professor Dr. Saw Win and hospital administrative committee for the permission to collect data at Parami General Hospital. We also grateful to the children and parents for their kind participation in our study, and the advisors from Institute of Nutrition, Mahidol University for valuable advice.

REFERENCES

1) Shrimpton R, Rokx C. The Double Burden of Malnutrition: A Review of Global Evidence: Health, Nutrition: and Population Discussion Paper. 2012.

2) Unicef. Levels and trends in child malnutrition: eSocialSciences. 2018.

3) Hartman C, Shamir R, Hecht C, Koletzko B. Malnutrition screening tools for hospitalized children. Curr Opin Clin Nutr Metab Care 15: 303-09. 2012.

4) Hecht C, Weber M, Grote V, Daskalou E, Dell'era L, Flynn D, Gerasimidis K, Gottrand F, Hartman C, Hulst J. Disease associated malnutrition correlates with length of hospital stay in children. Clin Nutr 34: 53-59. 2015.

5) Mehta N M, Corkins M R, Lyman B, Malone A, Goday P S, Carney L, Monczka J L, Plogsted S W, Schwenk W F, Parenteral A S F, Directors E N B O. Defining pediatric malnutrition: a paradigm shift toward etiology□related definitions. Journal of Parenteral and Enteral Nutrition 37: 460-81. 2013.

6) Hulst J M, Zwart H, Hop W C, Joosten K F. Dutch national survey to test the STRONGkids nutritional risk screening tool in hospitalized children. Clin Nutr 29: 106-11. 2010.

7) Agostoni C, Axelsson I, Colomb V, Goulet O, Koletzko B, Michaelsen K F, Puntis J W, Rigo J, Shamir R, Szajewska H. The need for nutrition support teams in pediatric units: a commentary by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 41: 8-11. 2005.

8) Kondrup J, Allison S P, Elia M, Vellas B, Plauth M. ESPEN guidelines for nutrition screening 2002. Clin Nutr 22: 415-21. 2003.

9) Huysentruyt K, Alliet P, Muyshont L, Rossignol R, Devreker T, Bontems P, Dejonckheere J, Vandenplas Y, De Schepper J. The STRONGkids nutritional screening tool in hospitalized children: a validation study. Nutrition 29: 1356-61. 2013.

10) Organization, W.H., WHO child growth standards: length/height for age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age, methods and development. 2006: World Health Organization.

11) Organization W H. Training course on child growth assessment. Geneva: WHO: p17-25. 2008.

12) Organization, W.H., WHO Anthro (version 3.2. 2, January 2011) and macros. Geneva: WHO. 2011.

13) Barba C V, Cabrera M I Z. Recommended dietary allowances harmonization in Southeast Asia. Asia Pac J Clin Nutr 17: 405-08. 2008.

14) Becker P, Carney L N, Corkins M R, Monczka J, Smith E, Smith S E, Spear B A, White J V, Nutrition A O, Dietetics, Parenteral A S F, Nutrition E. Consensus statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition: indicators recommended for the identification and documentation of pediatric malnutrition (undernutrition). Nutr Clin Pract 30: 147-61. 2015.

15) Landis J R, Koch G G. The measurement of observer agreement for categorical data. Biometrics: 159-74. 1977.

16) Bujang M, Baharum N. Guidelines of the minimum sample size requirements for Cohen's Kappa. Epidemiol Biostatistics Public Health 14: e12267-61. 2017.

17) Joosten K F, Hulst J M. Nutritional screening tools for hospitalized children: methodological considerations. Clin Nutr 33: 1-5. 2014.

18) Wonoputri N, Djais J T, Rosalina I. Validity of nutritional screening tools for hospitalized children. J Nutr Metab 15: 1-6. 2014.

19) Mueller C, Compher C, Ellen D M, Parenteral A S F, Directors E N B O. ASPEN clinical guidelines: nutrition screening, assessment, and intervention in adults. Journal of Parenteral and Enteral Nutrition 35: 16-24. 2011.

20) Joosten K, Van Der Velde K, Joosten P, Rutten H, Hulst J, Dulfer K. Association between nutritional status and subjective health status in chronically ill children attending special schools. Qual Life Res 25: 969-77. 2016.

21) Rub G, Marderfeld L, Poraz I, Hartman C, Amsel S, Rosenbaum I, Pergamentzev-Karpol S, Monsonego-Ornan E, Shamir R. Validation of a nutritional screening tool for ambulatory use in pediatrics. J Pediatr Gastroenterol Nutr 62: 771-75. 2016.

22) Gupta M, Jindal R. Assesment of Nutritional Status of Under Five Children Attending Outpatient Departement At a Tertiary Care Hospital: A Study from North India. International Journal of Scientific Research and education (IJSRE) 4: 5283-87. 2016.

23) Gholampour Z, Hosseininasab M, Khademi G, Sezavar M, Abdollahpour N, Imani B. Assessment of nutritional status based on STRONGkids tool in Iranian hospitalized children. Int J Child Health Nutr 4: 61-6. 2015.

ORIGINAL

Carbohydrate Profile, Total Dietary Fiber and *In vitro* Glycemic Index of Taro- Corn and Sweet Potato- Corn noodles.

Hazel T. Lat[1*], Wilma A. Hurtada[2], Erlinda I. Dizon[3], Aimee Sheree A. Barrion[2]

[1]*Department of Science and Technology-Food and Nutrition Research Institute, Bicutan, Taguig City, Philippines.*
[2]*Institute of Human Nutrition and Food, College of Human Ecology, University of the Philippines Los Baños, Philippines.*
[3]*Institute of Food Science and Technology, Food Science Cluster, College of Agriculture, University of the Philippines Los Baños, Philippines.*
(received January 30, 2019)

ABSTRACT *Background.* The carbohydrate profile and *In vitro* glycemic index of taro, sweet potato and IPB var. 6 white corn flours and the noodles from taro-corn and sweet potato-corn flour mixtures were studied. *Results*. Processing the flours into noodles significantly affected its carbohydrate profile (starch and amylose), total dietary fiber and glycemic index. There was a significant reduction ($p<0.05$) in the starch content when the flours were processed into noodles, but a significant increase ($p<0.05$) in the amylose content, total dietary fiber and glycemic index when the flours were processed. *Conclusion*. The results of this study may provide opportunities to promote and support the use of the taro, sweet potato and IPB var. 6 white corn flours into processed foods like noodles which can help improve its production and utilization potentials.

Keywords: Taro; Sweet Potato; Corn; Dietary Fiber; Glycemic Index

INTRODUCTION

Carbohydrates, such as rice, breads, and noodles are the major sources of food, because it provides most of the daily energy needs. Among these, noodles are popularly known for their ease of preparation, availability and deemed as a cheap source of carbohydrates. Even in disaster situations, noodles are usually being provided as a source of nourishment. Noodles are usually prepared using ingredients such as wheat flour, egg and salt. These ingredients provide high energy, but most often, it does not contain other nutrients that are beneficial for growth and development. That is why, noodles in the country are starting to be fortified with vitamins and also have additional vegetable ingredients. Although, it will be good to try substituting wheat flour with indigenous flours, for added nutritional and health benefits, such as taro flour, sweet potato flour, and corn flour.

Tubers and root crops, such as taro, sweet potato and crops such as corn or maize, are basically important sources of carbohydrates as an energy source and as a staple in the tropical and sub-tropical countries (1). These products have nutritionally beneficial components high dietary fiber content and have a lower glycemic index value (2).

Dietary fiber is an important part of a healthy diet. It is the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine (3), while the glycemic index of foods was introduced to provide additional information about foods and to classify them in terms of their glycemic response whether foods eaten release glucose rapidly or in a slow and sustained fashion for a period of time (4). From a nutrition perspective, it is not particularly important to know the exact GI value of every food as this will change depending on various factors. What is important is to be able to identify which foods are absorbed over a longer period of time (low GI; GI <55), which foods elicit a rapid surge of glucose (energy) into the bloodstream (high GI; GI > 70), and which are in between (intermediate GI; GI 55-70). Consequently, foods high in carbohydrates can thus be arranged into these broad categories to guide dietary choices (5).

This study specifically determined the carbohydrate profile (starch and amylose), total dietary fiber content and in vitro glycemic index of the taro [Colocasia esculenta (L.) Schott], sweet potato [Ipomoea batatas (L.) Poir], IPB var. 6 white corn [Zea mays L] flours, and the cooked and uncooked taro-corn and sweet potato-corn noodles to determine the effect of processing. The results of this study may provide opportunities to promote and support the use of the taro, sweet potato and IPB var. 6 white corn flours into processed foods like noodles which can help improve its production and utilization potentials.

This study was conducted at IHNF, UPLB from May to June 2014.

*To whom correspondence should be addressed: lat.hazel@gmail.com

METHODS

Experimental Materials

Quality Protein Maize (IPB var. 6) and Sweet Potato (NSIC 31 variety) were obtained from the Institute of Plant Breeding, College of Agriculture, University of the Philippines Los Baños, Laguna. Taro corms (NSIC V9 variety) were obtained from the Philippine Root Crop Research and Training Center, Visayas State University (VSU), Visca, Baybay City, Leyte.

Processing of taro corms and sweet potato roots

The raw taro corms and sweet potato roots underwent different physical treatments such as washing, peeling, dicing, soaking, drying and milling before they were processed into taro and sweet potato flour. The taro and sweet potato flour was then mixed with the IPB var. 6 white corn flour and was used in the preparation of the noodles. It was analyzed for its carbohydrate profile, total dietary fiber content and *in vitro* glycemic index. All the chemical analyses were done at the Analytical Laboratory of the Institute of Human Nutrition and Food at the University of the Philippines Los Baños.

Preparation of the taro-corn and sweet potato-corn noodles

The taro, sweet potato and IPB var. 6 white corn flours were used in the preparation of the noodles. The recipes were standardized to determine the right proportion of the ingredients that yielded the most acceptable product.

Selection of flour proportion for noodle production

Preliminary study was conducted to produce flat noodles from the different possible combinations of the taro-corn flour and sweet potato-corn flour (100:0, 90:10, 80:20,70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100). The 70:30 taro-corn and 70:30 sweet potato-corn noodles were both slightly liked for their aroma, texture and flavour, and was acceptable as compared to the other proportions.

Chemical analyses

Starch content was determined using the anthrone method while the amylose content was determined using a colorimetric assay by Williams *et al*. (6).

Total Dietary Fiber

The total dietary fiber was determined using the enzymatic-gravimetric method using the total dietary fiber assay kit (TDF-100 A and TDF – C10) of Sigma-Aldrich, Inc.

Estimation of Glycemic Index

The kinetics of in vitro starch digestion was followed by a nonlinear model established by Goni *et al.* (7).

Statistical analysis

Results of the chemical tests were analyzed using ANOVA and LSD. All the statistical tests were performed at 5% level of significance.

RESULTS AND DISCUSSION

Carbohydrate Profile

The taro flour has the highest starch content at 75.22 % followed by the sweet potato flour at 71.45 % and the IPB var. 6 white corn flour at 70.98 %. As for the noodles, the sweet potato-corn uncooked noodles have higher starch content at 65.32 % as compared to the uncooked taro-corn noodles at 52.79 %. There was a significant decrease in the starch content when the noodles were cooked, from 52.79 % starch content of the uncooked taro-corn noodles to 30.91 % in the cooked taro-corn noodles, as well as in the sweet potato-corn noodles at 65.32 % for the uncooked noodles to 55.42 % in the cooked noodles (Table 1).

Table 1. Starch and amylose content of taro, sweet potato, IPB Var. 6 flour and taro-corn and sweet potato-corn noodles

Sample	Starch (%)	Amylose (%)
Taro Flour	75.22 ± 1.30^{a}	39.75 ± 2.33^{b}
Sweet Potato Flour	71.45 ± 2.24^{b}	18.57 ± 0.21^{f}
Quality Protein Maize Flour	70.98 ± 0.62^{b}	30.45 ± 0.21^{e}
Taro-Corn uncooked noodles	52.79 ± 0.76^{d}	33.52 ± 0.58^{d}
Taro-Corn noodles	46.80 ± 0.91^{e}	36.27 ± 1.07^{c}
Sweet potato-Corn uncooked noodles	65.32 ± 1.96^{c}	39.05 ± 1.15^{b}
Sweet potato-Corn cooked noodles	55.42 ± 1.82^{f}	45.14 ± 2.21^{a}

Means that share the same letter within columns are not significantly different at p (<0.05).
Waxy (0-5% amylose); very low (5-12% amylose); low (12-20%); intermediate (20-25%); high (25-33%)

The taro flour has the highest amylose content at 39.75 % followed by the IPB var. 6 white corn flour at 30.45 % and the sweet potato flour at 18.57 %. For the noodles, there was a significant increase in the amylose content after cooking, from 33.52 to 36.27 % in the taro-corn noodles and from 39.05 % amylose content in the uncooked sweet potato-corn noodles to 45.14 % after cooking. Also, the amylopectin content was measured. The sweet potato flour has the highest amylopectin content at 40.53 % followed by the IPB var. 6 white corn flour and the taro flour at 40.53 and 35.47 % respectively.

The amylose content in starch is one of the important factors influencing starch pasting and the strength of starch gel due to its quick retrogradation, association and interaction to lipids, and amylopectin form helical complex giving strong gel structures. Starches with high amylose content were desired for manufacture of starch noodles (8). Taro has a smooth-textured gel, which would be suitable for noodle processing, which requires a smooth mouth feel and avoidance of grainy texture. Also, past studies have shown that sweet potato flour may potentially be used in noodle processing and bread production (2). However, it was also reported that high amylose corn starch was not suitable for noodle making because it was not sufficiently gelatinized at atmosphere condition, leading to almost no amylose molecules released to participate into the noodle structure formation (8). As what was observed in the preliminary study wherein the 100% corn flour cannot be formed into noodles. However, if mixed into starches with high amount of protein, lipid and ash, which can reduce the clarity of starch paste and hinder the swelling of the starch granule because of the high rate formation of amylose-lipid complex. Nevertheless, the protein and lipid played important role in retention of amylose in starch noodles during cooking, resulting in minimizing cooking loss (8).

Total Dietary Fiber

Among the three flours, the sweet potato flour has the highest total dietary fiber content at 4.74 %, followed by the IPB Var.6 white corn flour and the taro flour at 2.71 % and 2.63 % respectively. The total dietary fiber content of the cooked noodles increased from, 5.02 % to 5.36 % in the cooked taro-corn noodles while the uncooked sweet potato-corn noodles also increased from 7.21 % to 10.17 % in the cooked sweet potato-corn noodles (Table 2). This could have been due to the fact that an increase in temperature leads to a breakage of weak bonds between polysaccharide chains. Also, glycosidic linkages in the dietary fiber polysaccharides may be broken (9). According to Dhingra *et al.* (10), grinding may affect the hydration properties, in particular, the kinetics of water uptake as the result of the increase of surface area, the fiber hydrates more rapidly. Heating generally changes the ratio of the soluble to insoluble fiber. Simple processes, such as soaking and cooking tend to modify the composition and availability of nutrients. This may also modify the plant cell wall material that may have physiological effects. In a study done in wheat bran, it has been found out that thermal treatment, such as boiling, cooking or roasting-originate an increase of total fiber that is not due to new synthesis but rather to the formation of fiber-protein complexes that are resistant to heating and are qualified as dietary fiber. According to Pushparaj and Urooj (11) changes in dietary fiber composition of processed cereal and pulses have been reported where increase in the total dietary fiber content could be due to formation of resistant starch.

The amount of total dietary fiber is important because of its influence on glucose absorption, post-prandial glucose levels, and glycemic index (12).

Table 2. Total dietary fiber content

Sample	Total Dietary Fiber (%)
Taro Flour	2.63 ± 0.21^{d}
Sweet Potato Flour	4.74 ± 0.59^{c}
Quality Protein Maize Flour	2.71 ± 0.21^{d}
Taro-Corn uncooked noodles	5.02 ± 0.24^{c}
Taro-Corn noodles	5.36 ± 0.68^{c}
Sweet potato-Corn uncooked noodles	7.21 ± 0.86^{b}
Sweet potato-Corn cooked noodles	10.17 ± 0.68^{a}

Means that share the same letter within columns are not significantly different at p (<0.05)

Glycemic Index

The classification of foods based on their glycemic index has dispelled the repeatedly suggested dietary notion that carbohydrate-rich foods have deleterious health effects, and as such, consumption should be limited. In fact, there are numerous evidence-based studies which dismiss the negative view of carbohydrate-rich foods and clearly demonstrate that "not all carbohydrates are created equal". Furthermore, variations in the physicochemical properties of complex carbohydrates have been shown to elicit dissimilar physiological effects when consumed. Also, some complex carbohydrate-rich foods are undeniably beneficial and do not cause blood glucose levels to spike any greater than simple sugars. However, food preparation is important and should be considered, as the method of cooking can alter the structure and the nature of the starches (13).

Enzyme hydrolysis of starch molecules to produce glucose is important to provide energy for plant metabolism, food, feeds and ethanol production. The starch granules are hydrolyzed at a slower rate by enzymes than the gelatinized, amorphous starch molecules. The susceptibility of the starch granules depends on the granular size, the structure of the amylopectin, the amylose content, the lipid content, and the reaction pattern of the enzyme. Generally, the larger the starch granule, are normally digested at a slower rate than the smaller starch granules because the larger granules have a smaller relative surface area for enzyme hydrolysis (14).

White bread, used as reference in this study, showed a digestion value of 75.3 % at 180 minutes (Figure 1), which resembles that reported by Goñi *et al.* (7), but previous works, such as the one done by Grandfelt *et al.* (15) as reported by Tovar *et al.* (2002) have found that white bread showed a digestion value of 50 %. This could be explained, due to the different methodologies that were used, such as the inclusion of pepsin in the hydrolysis and the use of an unrestricted system (7).

Among the flours, the IPB Var. 6 white corn flour has the lowest total starch hydrolysis rate at 29.13 % and the sweet potato flour has the highest rate at 51.66 % as compared to that of the white bread. The cooked taro-corn and sweet potato-corn noodles also showed a high starch hydrolysis rate of 67.05 % and 62.22 % respectively (Figure 1).

The course of hydrolysis differed greatly among various samples, being markedly slow in the case of IPB Var. 6 white corn flour, sweet potato flour, taro flour and the uncooked taro-corn noodles and sweet potato-corn noodles, but much more rapid in the case of white bread and that of the cooked taro-corn and sweet potato-corn noodles, which showed higher hydrolysis index. However, it should be kept in mind that ingredients included in the noodle formulation might have also affected starch digestibility (16).

The rate of starch hydrolysis in the samples varied widely suggesting that other factors other than hydrolytic enzyme activity. It can be observed that the cooked noodles hydrolyzed more rapidly as compared to the uncooked noodles. This is because cooking greatly increased the rate at which starch can be hydrolyzed by gelatinizing the starch and making it more readily available for enzymatic attack (17). The degree of starch gelatinization is not the only factor that affects susceptibility of starch to enzyme action. The digestibility of starch can be affected by several factors including the composition and physical form of the starch, protein-starch interactions, the physical form of the food, the integrity of the starch-containing cells, and presence of anti-nutritional factors (18), while the lower levels of starch hydrolysis that were observed in the taro, sweet potato and IPB var 6 white corn flour, may be attributed to the presence of cell wall and other components such as the dietary fiber and mucilage (19) as what was observed by Bordoloi *et al.* (20) in the starch of cooked potatoes.

Table 3 shows that the reference sample, white bread has an estimated glycemic index of 94.61. For the flour samples, the sweet potato flour has the highest glycemic index at 77.20, followed by the taro flour and the IPB Var 6 white corn flour at 65.46 and 60.79 respectively. For the noodles, cooking showed a decrease in the glycemic index of the taro-corn and sweet potato-corn noodles at 84.92 for the uncooked taro-corn noodles to 72.24 for the cooked taro-corn noodles, and the uncooked sweet potato-corn noodles at 88.43 to 74.45 for the cooked sweet potato-corn noodles.

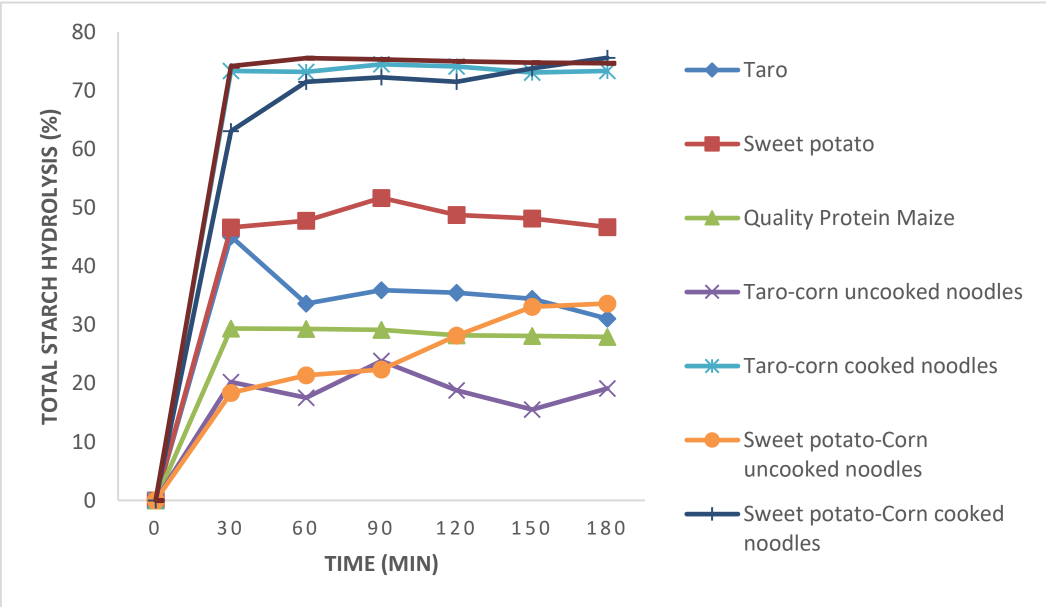

Figure. 1. Average hydrolysis curves of starch in taro flour, sweet potato flour, IPB var. 6 white corn flour (QPM), and cooked and uncooked taro-corn and sweet potato-corn noodles.

Table 3. Starch hydrolysis and estimated glycemic index (EGI) of flour and noodles

SAMPLE	H_{90}	$C\infty$	k	HI	EGI
White Bread	75.30 ± 1.19^{a}	79.29^{a}	0.03	100 ± 0.00^{a}	94.61 ± 0.00^{a}
Taro Flour	35.91 ± 3.41^{bd}	39.34^{bd}	0.03	46.91 ± 6.11^{bd}	65.46 ± 3.35^{bd}
Sweet Potato Flour	51.66 ± 1.99^{c}	55.46^{c}	0.03	68.28 ± 3.80^{ch}	77.20 ± 2.08^{ch}
Quality Protein Maize Flour	29.13 ± 5.13^{d}	32.31^{d}	0.03	38.38 ± 6.71^{d}	60.79 ± 3.70^{d}
Taro-Corn uncooked noodles	62.22 ± 1.85^{eh}	66.04^{ec}	0.03	82.34 ± 0.95^{e}	84.92 ± 0.52^{e}
Taro-Corn noodles	44.88 ± 0.82^{fbc}	48.43^{fbc}	0.03	59.25 ± 2.01^{fh}	72.24 ± 1.10^{fh}
Sweet potato - corn uncooked noodles	67.05 ± 4.12^{ga}	70.93^{gae}	0.03	88.74 ± 4.58^{g}	88.43 ± 2.52^{g}
Sweet potato – corn cooked noodles	47.89 ± 2.69^{hc}	51.52^{hcf}	0.03	63.27 ± 4.25^{h}	74.45 ± 2.33^{h}

Means that share the same letter within columns are not significantly different at p (<0.05)
GI Classification: Low GI <55; intermediate 55-70; High >70;
H_{90} – Starch hydrolysis at 90 minutes; $C\infty$ - Equilibrium concentration; k- Kinetic Constant; HI- Hydrolysis Index

The sweet potato flour exhibit the highest GI value among the three flours at 77.20 and also the lowest amylose content at 18.57 %, while the taro flour and the IPB var. 6 exhibited an intermediate GI value at 65.46 and 60.79 respectively, and also exhibited a higher amylose content than that of the sweet potato flour at 39.75 and 30.45 % for taro and IPB var. 6 white corn flour respectively. When the flours are made into noodles, it resulted in higher amylose content for the sweet potato-corn uncooked and cooked noodles at 39.05 to 45.14 % respectively. As for the taro-corn noodles, when the noodles were cooked it resulted in a higher amylose content at 33.52 % for the uncooked noodles to 36.27 % for the cooked noodles. When the noodles are cooked, the amylose content increases, but the GI value decreased, from the GI value of 84.92 to 72.24 for the taro-corn noodles and the GI value of 88.43 to 74.45 for the sweet potato-corn noodles (Figure 1). Bahado-Singh *et al.* (13) reported that rice with high amylose content was accompanied by a lower metabolic response to and lower GI values. Also, boiling lowers GI values. This may be link to the chemical structure of starches - that is the amylose-amylopectin ratio (21). The amylose content is known to be negatively correlated with the starch susceptibility to amylase hydrolysis (22). Juliano (23) suggested the classification of amylose content in rice as waxy (0-5 %), very low (5-12 %), low (12-20 %), intermediate (20-25 %) and high (25-33 %). Waxy starch granules are more susceptible to enzyme hydrolysis than intermediate starch granules, and the intermediate starch granules are more susceptible to enzyme hydrolysis than high-amylose starch granules (24-25). Concomitantly, greater amounts of resistant starches may have been retained in the boiled foods. Furthermore, as these foods cool, the possibility of forming resistant starches increases. This occurs as the starches undergo recrystallization due to the formation of the intermolecular hydrogen bonds. Also, these resistant starches, after the leaching of free sugars during the boiling process also play a role in retarding the enzymatic degradation of the starches, thus reducing the glycemic response (13). Also, apart from the amylose content, high dietary fiber was believed to reduce the blood glucose response, and thus lower the GI value of a food. Dietary fiber influences the starch digestibility by changing the microstructure of foods, which decreases the susceptibility of starch to amylolytic attack, and by limiting water availability, which restricts starch gelatinization (26).

The sweet potato flour has the highest total dietary fiber content and the highest GI value among the three flours, followed by the taro and the IPB Var. 6 QPM white corn flour (Figure 2). This was because grinding has an effect on the gross matrix structure of the flour. The grinding process increases the glycemic response and glycemic index of the flours (27). The uncooked sweet potato-corn noodles has a lower dietary fiber content but have a higher GI value as compared to the cooked sweet potato-corn noodles. The same can be observed in the taro-corn noodles wherein there was an increase in the dietary fiber content and a decrease in the GI value after cooking. This might be due to the higher amylose content of the cooked noodles as compared to the uncooked noodles. This is due to high amylose slowed the digestion rate. There is greater hydrogen bonding between glucose units in amylose molecule than the amylopectin molecule, thus less exposure to enzymatic digestion (28).

Wolever (29), studied naturally present fiber contents in foods and their subsequent glycemic response, and revealed that the total dietary fiber was significantly related to the glycemic index of foods. This was supported by the study done by Brouns *et al.* (27), Riccardi *et al.* (30) and Allen *et al.* (12), that stated that foods that are rich in fiber generally have a low glycemic index, although not all foods with a low glycemic index necessarily have high fiber content, as what have been observed between the glycemic index values and the total dietary fiber values of the uncooked and cooked noodles.

Thus, the presence of amylose and dietary fiber in the different samples used affects its glycemic index. The combined effect of amylose and dietary fiber contributed in the low glycemic index value in the cooked taro-corn and sweet potato-corn noodles.

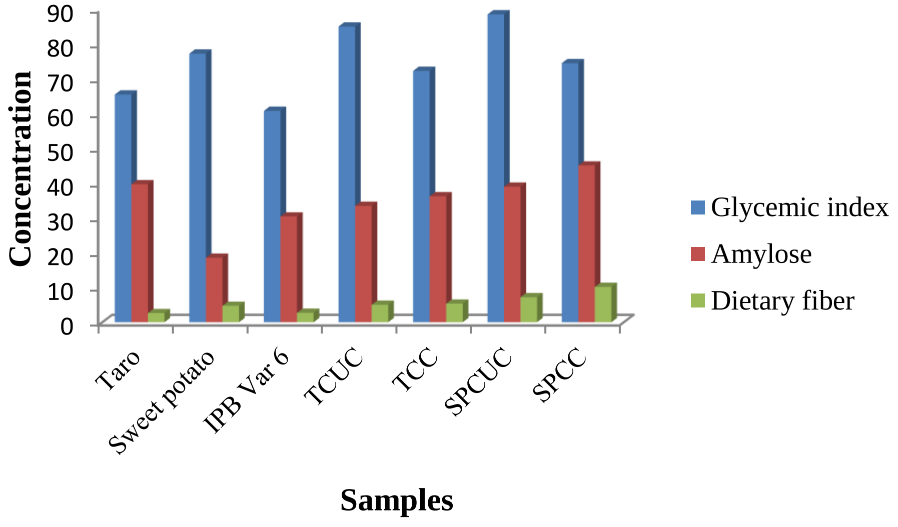

Figure 2. Comparison between the glycemic index, amylose content and total dietary fiber for each sample.

CONCLUSION

The results of the study revealed that processing the taro flour, sweet potato flour, IPB var.6 white corn flour, and the uncooked and cooked taro-corn and sweet potato-corn noodles significantly affected the carbohydrate profile, specifically, the starch and amylose content, the total dietary fiber content and the glycemic index. Boiling the noodles significantly decreased its starch content, and its glycemic index, while significantly increasing its amylose content and total dietary fiber content.

Studies on the shelf life and effect of storage temperature to the flours, as well as the noodles must be done. Another area of interest would be the utilization of the taro and sweet potato flours as an ingredient in other products to improve its palatability, especially for children. Also further studies regarding the cost analysis/profitability in the production of the flours, and its products, like the noodles should be pursued. Lastly, the use of the taro-based and sweet potato-based products in feeding programs should be tested to know whether it can help improve the nutritional status, and thus preventing malnutrition.

ACKNOWLEDGEMENT

Special thanks to the Department of Science and Technology- Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP) for the full financial support and scholarship grant; to Dr. Dilberto Ferraren of Philippine Root Crop Research and Training Center, Visayas State University, Visca, Baybay City, Leyte, for providing the taro samples, and to Prof. Felicito Rodriguez of the Institute of Plant Breeding and Institute of Human Nutrition and Food, University of the Philippines, Los Baños for providing the sweet potato samples.

REFERENCES

1) Liu Q, Donner E, Yin Y, Huang RL, Fan MZ. The physicochemical properties and in vitro digestibility of selected cereals, tubers, and legumes grown in China. Food Chemistry 99: 470-477. 2006.
2) Aprianata A, Purwandari U, Watson B, Vasiljevic T. Physico- chemical properties of flours and starches from selected commercial tubers available in Australia. International Food Research Journal. 16: 507-520.2009.
3) [AACC] American Association of Cereal Chemist. The Definition of Dietary Fiber. Cereal Foods World. Vol 46, No 3:112-126. 2001.
4) Jenkins DJA, Thomas DM, Wolever TMS. Glycemic Index of Foods: A Physiological Basis for Carbohydrate Exchange. Am J Clin Nutr. 34: 362-366. 1981.
5) Gibson N. Development of a Rapid Assessment Method for the Glycaemic Index. Master's Thesis. University of Pretoria. 2010.
6) Williams VR, Wu WT, Tsai TY, Bates HG. Varietal differences in amylase content of rice starch. J. Agr. Food Chem. 6:47-48.1958.
7) Goñi I, Garcia-Alonso A, Saura-Calixto F. A Starch Hydrolysis Procedure to Estimate Glycemic Index. Nutrition Research. Vol 17, No.3:427-437. 1997.
8) Thao HM, Noomhorm A. Physicochemical properties of sweet potato and mungbean starch and their blends for noodle production. J Food Process Technol 2:1.2011.
9) FAO. Carbohydrates in Human Nutrition. Agriculture and Consumer Protection. Rome:WHO. 1997.
10) Dighra D, Michael M, Rajput H and Patil RT. Dietary foods: A review. J Food Sci Technol. 49 (3):255-266. 2012.

11) Pushparaj FS, Urooj A. PUSHPARAJ FS, 2011. Influence of processing on dietary fiber, tannin, and in vitro protein digestibility of pearl millet. Food and Nutrition Sciences. 2:895-900. 2011.
12) Allen JC, Corbitt AD, Maloney KP, Butt MS, Truong VD. Glycemic index of sweet potato as affected by cooking methods. The Open Nutrition Journal 6, 1-11. 2012.
13) Bahado-Singh PS, Riley CK, Wheattey AO, Lowe HIC. Relationship between processing method and the glycemic indices of ten sweet potato (Ipomoea batatas) cultivars commonly consumed in Jamaica. J Nutr Metab. 2011:584832. 2011.
14) Tester RF, Karkalas J, Qi X. Starch Structure and digestibility Enzyme-Substrate Relationship. World's Poult Sci J. 60:185-195. 2004.
15) Grandfelt Y. Food Factors Affecting Metabolic Responses to Cereal Products. PhD Dissertation. University of Lund: Sweden.1994.
16) Tovar J, Sayago-Ayerdi SG, Peñalver C, Paredes-Lopez O and Bello-Perez LA. In vitro starch hydrolysis index and predicted glycemic index of corn tortilla, black beans (Phaseolus vulgaris L.) and Mexican "taco". Cereal Chem. 80 (5): 533-535.2002.
17) Snow P, O'dea K. Factors Affecting the Rate of Hydrolysis of Starch in Food. Am J Clin Nutr. 34:2721-2727.1981.
18) Siller ADCP. In Vitro Starch Digestibility and Estimated Glycemic Index of Sorghum Products. Master's Thesis. Texas A & M University. Available at <http://repository.tamu.edu>. Accessed 13 November 2014.
19) Mbofung CMF, Aboubakar YN, Njintang A, Abdou B, Baalam F. Physicochemical and Functional Properties of Six Varieties of Taro (Colocasia esculenta (L.) Schoot) Flour. J. Food Tech. 4(2): 135-142. 2006.
20) Bordoloi A, Jaspreet S, Kaur L. In vitro digestibility of starch in cooked potatoes as affected by guar gum: microstructural and rheological characteristics. Food Chemistry 133;1206-1213.2012.
21) Bjork I, Grandfelt Y, Liljeberg H, Tovar J, Ng ASP. Food Properties Affecting the Digestion and Absorption of Carbohydrates. Am J clin Nutr. 59(3). 1994.
22) Hasjim J. Enzyme Digestibility of Starch and Methods to Produce Enzyme- Resistant Starch to Improve Human Health. PhD Thesis. 2009. Iowa State University.Available at <http://lib.dr.iastate.edu/etd>. Accessed on 12 November 2014.
23) Juliano BO. Grain Quality of Philippine Rice. Muñoz Nueva Ecija, Philippines: 70pp Philipp Rice Res Inst.1992.
24) Fuwa H, Nakajima M, Hamada A, Glover DV. Comparative Susceptibility to Amylases of Starches from Different Plant Species and Several Single Endosperm Mutants and their Double-Mutant Combinations with Opaque-2 Inbred Oh43 Maize. Cereal Chem. 54:230-237.1977.
25) Jane J, Ao Z, Duvick SA, Wiklund M, Yoo SH, Wong KS, Gardener C. Structures of Amylopectin and Starch Granules: How are they synthesized. J Appl Glycosci. 50:167-172. 2003.
26) Kim HJ, White PJ. In vitro digestion rate and estimated glycemic index of oat flours from typical and high β-glucan oat lines. J Agric Food Chem. 60. 5237-5242.2012.
27) Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, Wolever TMS. Glycaemic index methodology. Nutrition Research Reviews. 18, 145-171. 2005.
28) Shanita SN, Hasnah H, Khoo CW. Amylose and Amylopectin in Selected Malaysian Foods and its Relationship to Glycemic Index. Sains Malaysiana. 40 (8): 865-870. 2011.
29) Wolever TMS. Relationship between dietary fiber content and composition in foods and the glycemic index. Am J Clin Nutr. 51:72-5. 1990.
30) Riccardi G, Rivellese AA, Giacco R. Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. Am. J. Clin. Nutr. Vol. 87 no. 1 269S- 274S. 2008.

ORIGINAL

Effects of Pre-Germinated Brown Rice on Blood Glucose Concentration of Type 2 Diabetes Mellitus Patients in Vietnam

Bui Thi Nhung[1], Le Dang Tuyen[1], Nguyen Do Van Anh[1], Nguyen Thi Hong Ha[2], Vu Thi Minh Thuc[3], Vu Anh Linh[2], Kei Yui[4], Yukihiko Ito[4], Shigeru Yamamoto[2*]

[1]*National Institute of Nutrition, Hanoi, Vietnam*
[2]*Asian Nutrition and Food Culture Research Center, Jumonji University, Saitama, Japan*
[3] *National ENT Hospital, Hanoi, Vietnam*
[4]*FANCL Research Center, Yokohama, Japan*

(received December 24, 2019)

ABSTRACT In recent years, type 2 diabetes mellitus (DM) prevalence in Vietnam is increasing rapidly. Newly diagnosed Vietnamese DM patients were reported to have low body mass index (BMI) of around 23, and high white rice intake has been identified as one of the major causes. As a recommended staple food substitute for white rice, brown rice has the disadvantages of hard texture and unfavorable taste. These drawbacks can be compensated in the process of making pre-germinated brown rice (PGBR), by soaking BR in lukewarm water to make it slightly sprouted. Previous studies have suggested that similar to BR, PGBR is effective in modulating blood glucose and lipid concentration levels. In this study, a 4-month PGBR administration was applied to evaluate the efficiency of PGBR on HbA1c index of Vietnamese type 2 diabetics. The present results suggest that replacing WR with PGBR may be useful in modulating blood glucose levels in Vietnamese type 2 DM patients.

Keywords: PGBR; DM; HbA1c; Vietnamese

INTRODUCTION

The prevalence of type 2 diabetes mellitus (DM) is increasing worldwide at a dramatic pace. According to the World Health Organization (WHO), DM ranked 8th among 10 leading causes of death in the world, having caused 1.5 million deaths within a year of 2013 (1). Around 80% of people with diabetes reside in developing nations, where striking transformation in lifestyle, population structure and growth rates are major contributors to the wide spread of the epidemic. As reported by the International Diabetes Federation (2), the prevalence of type 2 DM in Vietnam has doubled within a decade, rising from 2.7% (2002) to 5.4% (2012). As of 2012, Vietnam was home to nearly 3.3 million DM cases, with more than 54,000 deaths by DM-related diseases (2). It is anticipated that by 2030, the number of type 2 DM cases in Vietnam will double in comparison to 2010 (3).

Although DM has been consistently associated with obesity, studies have indicated that type 2 DM develops at a lower body mass index (BMI) range among Asians as opposed to those of Western populations, and that at the same BMI, Asian populations have a higher incidence of DM than their Western counterparts (4-6). In Vietnam, various studies in the 1990s and 2000s showed that Vietnamese DM patients have low BMI of approximately 23 (7-8). The use of white rice (WR) as a staple food may serve as the major factor, since it has been demonstrated and distinguished as a high glycemic index (GI) food and a risk factor for DM (10-11).

GI was proposed by Jenkins et al in 1980 as an indicator reflecting post-prandial blood glucose response after digesting foods (12). Since then, a strong relationship between GI and DM has been observed. It is expected that a carbohydrate-rich diet accompanied by rapid glucose absorption, i.e. a diet with high GI value, is strongly associated with increased risks for diabetes (13-14). Although it may vary depending on such factors as amylose content or refining process, the average GI value of WR is generally described to be higher than that of whole wheat cereals. In particular, GI value of WR was 76±7, 62±5 for BR, 30±9 for whole wheat and 29±5 for barley kernels in an earlier meta-analysis (15). A large-scale study with more than 59,000 subjects by Nanri et al linked high WR intake with an increased risk of type 2 DM in Japanese women (16). Although WR has been validated as a high GI food and a DM risk factor, for centuries it has been the major staple food that laid the foundation of the traditional Vietnamese diet. According to the National Nutrition Survey 2009-2010, as much as 67% of total energy intake in the diet of Vietnamese people comes from white rice, despite the dramatic change in dietary habits in recent decades (17). The long tradition of consuming rice as a staple

* To whom correspondence should be addressed: shigeruy@juminji-u.ac.jp

food makes it difficult for Asians, including the Vietnamese, to reduce rice intake.

As low GI foods, whole grains such as BR or whole wheat are often recommended to be consumed in combination with high GI staple foods in Asian style diets, namely WR and white bread, in order to restrain rapid elevation of blood glucose and control metabolic syndrome risk factors (18). Epidemiologic studies have demonstrated an inverse association between risks of metabolic syndrome and whole grain consumption. Ye et al (19) reviewed 66 papers published between 1996 and 2012 and reported a 21% decrease in DM and 21% decrease in heart diseases by the daily intake of 370g whole grains. Low GI diets have been known to exert positive effects similar to that of several anti-hyperglycemic pharmacological agents, as suggested by Brand-Miller in a previous meta-analysis (20). In recent years, significant evidence has transpired in respect of the influences of BR on blood glucose and DM risks. A cohort study by Harvard School of Public Health reviewing 3 studies on 197,228 subjects found that replacing 50g WR (equivalent to one third of a typical daily serving) with an alternative portion of BR everyday can help lower risk of type 2 DM by 16% (11).

BR is made by removing the outermost husk of the rice grain, whereas WR is made by depriving BR of its bran and germ portion. Due to the differences in processing, WR primarily consists of starch whilst BR maintains prominent nutrient contents of dietary fiber, vitamins and minerals (11, 21-22). BR however, has several problems of tough texture and unpleasant palatability, causing difficulty in digestion as well as meal preparation, especially in large quantity. In recent years, a new type of rice called pre-germinated brown rice (PGBR) has been developed and known to Vietnam. PGBR is produced by soaking BR in lukewarm water until slight germination. In PGBR, nutrient contents of BR are retained, whereas the hardness of the texture is reduced and the taste is considerably improved (23). Scientific evidence showed that the germination process also activates several bioactive components available in the rice bran like γ-aminobutyric acid (GABA), acylated steryl glycoside (ASG), or γ-oryzanols (21-23, 36). Similar to BR, PGBR is a low GI food (GI=54±5) (15).

PGBR has been reported to exert beneficial effects in preventing rapid elevation of blood glucose response and plasma insulin concentrations (21-22, 24). A previous study showed that a long-term diet with PGBR as a staple food reduced both blood glucose concentration and body weight in Vietnamese female subjects with impaired glucose tolerance (24).This study was conducted to evaluate the influence of PGBR consumption on the control of HbA1c value in DM patients under long-term anti-diabetic medication.

METHODS

Setting and study subjects

This study was designed as a parallel study in compliance with the Helsinki Declaration on Human studies. It was conducted from December 2014 to April 2015 in Nam Dinh Endocrinology Hospital of Nam Dinh province – a coastal administrative division located 90 km southeast from Hanoi, Vietnam. The population was approximately 2 million, nearly 18 per cent of which living in urban areas. The study protocol was approved by the Scientific Board and the Ethical Committee of Vietnam's National Institute of Nutrition (NIN).

Over the course of one month, medical database of more than two hundred DM patients affiliated with Nam Dinh Endocrinology Hospital were screened for subject selection. Patients referred to insulin injection were excluded. Based on the latest health-check and blood test results (with Hemoglobin A1c index of 6.5 – 9.5), 70 individuals were invited to participate. A briefing session was organized in order to provide potential participants with thorough details of the study, as well as the prospective beneficial health impacts of whole grains and PGBR. In addition, a test meal was provided to each person to account their ability of adaptation with PGBR as a staple food. Two weeks prior to the study baseline, informed consent was obtained from each individual. In accordance with the informed consent, participants were allowed to withdraw from the study at any time and by any reason. At the end of the subject selection process, a total number of 60 participants were achieved. To minimize the drop-out rate, subjects were contacted on weekly basis to track their rice intake and health status throughout the study.

All subjects have been taking oral anti-diabetic drugs for several years. Administration involves combination of common anti-hypoglycemic agents, particularly sulfonylureas (which act by increasing insulin secretion), intestinal α-glucosidase inhibitors (which delay the digestion and absorption of carbohydrates) and thiazolidinediones (which stimulate insulin activity without inducing insulin secretion).

Study design

This is a parallel study design. From 60 participants, 30 matched pairs were created according to age, HbA1c results and physical activity intensity. Matched pairs were then randomly divided into either WR group (control group) or PGBR group (intervention group).

Throughout the intervention period of 4 months, subjects were instructed to consume either WR or PGBR exclusively as a staple food. Before the beginning (baseline), at the midpoint (two months following baseline) and at the end of the study (final), anthropometric measurements, nutrition survey, and blood biochemical examinations were conducted. Participants were guided to pursue normal activities of daily life, as well as to continue anti-diabetic medication.

PGBR was produced in Nam Dinh Province with technological support from FANCL Corp., Japan. PGBR was prepared by soaking BR in lukewarm water of around 30°C in 22 hours and dried for preservation. The rice used in this study was of Tam Thom – a fragrant, long-grain rice variety native to the Red River Delta in Northern Vietnam. PGBR was provided to subjects of the PGBR group once every two weeks. Excluding staple food, participants were instructed to continue usual diet without any alteration.

Blood collection

Blood collection was conducted before the beginning of the study (baseline), at the midpoint and at the end of the study (final). Subjects were asked to fast for at least 10 hours before taking intravenous fasting blood samples in early morning. With aseptic precautions, each participant was subjected to a 6 mL blood withdrawal for the analyses of glucose, HbA1c, triacylglycerol (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C). Serum was separated by centrifuge and stored frozen at -30°C until analysis. Fasting glucose were done within 30 mins after blood collection by colorimetric method at Nam Dinh Endocrinology Hospital. Other analyses were carried out at Vietnam's NIN. Levels of TC, TG and HDL-C were measured by enzymatic colorimetric method. HbA1c was estimated by nephelometry method. Laboratory technologists were blinded to the identity of subjects and intervention status.

Anthropometric measurements

Weight, height, waist and hip circumferences and body fat percentage were measured three times, and the mean was used for analysis. Weight and height were measured while standing, wearing light clothing and without shoes. BMI was calculated as weight (kg) divided by the square of height (m^2). Waist circumference was measured mid-way between the umbilicus and the iliac crest, whereas hip circumference was measured at the widest circumference around the buttocks. Waist-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm). Body fat percentage was measured by the bioelectrical impedance method (HBF-351, Omron Co., Kyoto, Japan).

Nutrition survey

A nutrition survey for 3 non-consecutive days by the 24h recall method was implemented at baseline and final. During interviews, a full-size photo album of common food samples and standard household measures (cups, bowls, etc.) were used to facilitate the survey and increase the accuracy of food description. Energy and nutrient intake were calculated based on the Vietnamese Food Composition Table 2007. Fiber of PGBR was calculated using the data of a previous study on short grain rice (Japonica variety).

Statistical analysis

Qualitative variables were confirmed for normal distribution and compared using Student's *t*-test (paired and unpaired). Numerical data were presented as mean±SD. Values of $p<0.05$ were considered statistically significant for all analysis results. All statistical procedures were performed with SPSS software for Windows version 20.0 (SPSS, Inc., Chicago, IL).

RESULTS

Two participants from WR group and one from PGBR group withdrew from the study citing personal reasons. Data of two subjects were excluded for non-compliance. As a result, data of 55 subjects were presented in this study. Table 1 shows the physical characteristics and blood biochemical parameters of the subjects at baseline. At the outset of the study, except for hip circumference and footstep counts, no statistical differences were identified between two groups in age, weight, height, BMI, body fat percentage, waist circumference, WHR, fasting blood glucose, HbA1c, TG, TC, LDL-C and HDL-C.

Table 2 shows the comparison of physical characteristics and blood biochemical parameters of the PGBR and WR groups at baseline and final. Body fat percentage decreased significantly in both groups at final. Physical activity intensity (represented by footstep counts) of participants within each group remained constant during the time of intervention (p=0.206 in the PGBR and p=0.713 in the WR group). Glucose and HbA1c levels of the PGBR group at final remarkably declined in comparison with that at baseline, whereas in the WR group such changes were not detected.

Table 1. Comparison of physical characteristics and blood biochemical parameters between PGBR group and WR group at baseline.

Variables	PGBR (n=28)	WR (n=27)	p-value
Age (y)	61±9	59±7	0.32
Weight (kg)	60.06±8.96	57.82±10.38	0.395
Height (m)	1.58±0.08	1.57±0.08	0.713
BMI	23.9±2.39	23.2±2.88	0.328
Body fat (%)	30.0±6.3	29.34±6.6	0.698
Waist (cm)	85.8±7.4	84.0±6.5	0.343
Hip (cm)	91.3±4.9	88.6±4.8	0.043
Waist-hip ratio	0.94±0.6	0.95±0.36	0.584
Footstep counts	5339±2667	8251±4132	0.026
Glucose (mmol/L)	9.17±1.80	10.0±1.80	0.089
TC (mmol/L)	5.04±0.77	4.96±0.91	0.74
HDL-C (mmol/L)	1.3±0.135	1.31±0.16	0.643
LDL-C (mmol/L)	2.82±0.50	2.84±0.87	0.937
TG (mmol/L)	2.38±2.00	2.99±2.85	0.364
HbA1c (%)	6.92±0.36	7.03±0.46	0.349

Data are mean±SD. P-values obtained by unpaired t-test. TC: Total cholesterol. HDL-C: High density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, TG: triacylglycerol, HbA1c: hemoglobin A1c.

Table 2. Comparison of physical characteristics and blood biochemical parameters in PGBR group and WR group at baseline and final.

Variables	PGBR group (n=28)			WR group (n=27)		
	Baseline	Final	p-value	Before	Final	p-value
Weight (kg)	60.06±8.96	60.18±8.97	0.531	57.82±10.38	57.80±10.7	0.964
BMI	23.9±2.39	23.9±2.54	0.688	23.2±2.88	23.2±2.94	0.864
Body fat (%)	30.0±6.3	28.5±6.6	<0.001	29.34±6.6	26.98±6.66	<0.001
Waist (cm)	85.8±7.4	86.79±7.0	0.45	84.0±6.5	84.03±6.16	0.935
Hip (cm)	91.3±4.9	90.65±5.7	0.114	88.6±4.8	88.1±5.5	0.357
Waist-hip ratio	0.94±0.06	0.96±0.05	0.007	0.95±0.036	0.95±0.04	0.307
Footstep counts	5339±2667	6064±3279	0.206	8251±4132	7857±3707	0.713
Glucose (mmol/L)	9.17±1.80	7.96±2.3	0.021	10.0±1.80	9.73±2.83	0.639
TC (mmol/L)	5.04±0.77	4.75±0.69	0.05	4.96±0.91	4.74±0.71	0.296
HDL-C (mmol/L)	1.3±0.135	1.22±0.17	0.071	1.31±0.16	1.17±0.16	0.003
LDL-C (mmol/L)	2.82±0.50	2.84±0.51	0.851	2.84±0.87	2.87±0.57	0.798
TG (mmol/L)	2.38±2.00	1.75±0.64	0.083	2.99±2.85	1.87±0.92	0.029
HbA1c (%)	6.92±0.36	6.2±0.77	<0.001	7.03±0.46	7.12±0.84	0.626

Data are mean±SD. P-values obtained by paired t-test. TC: Total cholesterol. HDL-C: High density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, TG: triacylglycerol, HbA1c: hemoglobin A1c.

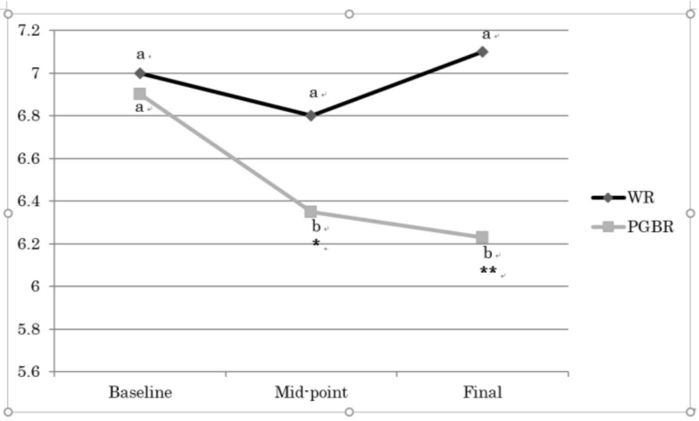

Figure 1. Changes in HbA1c

Different letters denote statistical significance within a group (p<0.05).

**, ** Significant differences between WR and PGBR groups at p<0.05 and p<0.001, respectively.*

Figure 1 shows the changes in HbA1c levels of the two groups during the time of intervention. Within one month and a half after the study implementation, HbA1c mean value of the PGBR group drastically declined from 6.9 to 6.5, which is the cut-off point for DM diagnosis as recommended by WHO. At the middle of the study, HbA1c levels of the PGBR group dropped to 6.4. Average HbA1c value of the PGBR group at the end of the study was 6.2, indicating a significant reduction compared to the baseline (p<0.001). Such decreases were not observed within the WR group (HbA1c 7.0 and 7.1 as initial and final values, respectively). In addition, no meaningful difference in HbA1c levels was detected among both groups at the beginning of the study (p=0.33). Between-group disparity emerged two months after the baseline (p=0.001) and was further emphasized at the final (p<0.001).

Table 3 shows the energy and nutrient intakes of the PGBR and WR groups at baseline and final. The intakes of energy, protein, lipid and carbohydrates between the two groups were similar. Mean fiber intake of the PGBR group at final was 10.9 g/day, significantly higher (p=0.05) than that of the WR group (7.7 g/day), despite the similarity between the two values at baseline.

Table 3. Energy and nutrient intakes of PGBR group and WR group at baseline and final.

Variables	Baseline			Final		
	PGBR (n=28)	WR (n=27)	p-value	PGBR (n=28)	WR (n=27)	p-value
Energy (kcal)	1485±381	1453±360	0.758	1448±329	1462±256	0.86
Carbohydrates (g)	228±63.7	237±65.5	0.63	237±57.4	231±59.4	0.689
Protein (g)	60.4±15.3	61.2±20	0.87	61±15.4	58.2±11.6	0.44
Lipid (g)	35.9±18.6	29.1±11.6	0.11	28.7±9.2	34±11.1	0.56
Dietary fiber (g)	7.9±4.8	7.9±3.2	0.959	10.9±4.6	7.7±3.2	0.05

p-values obtained by unpaired t-test

DISCUSSION

Despite medicine administration, HbA1c levels in the WR group barely changed during the time of the study. On the other hand, HbA1c levels of the PGBR group dropped drastically, and after two months of intervention, it was lower than the cut-off value for DM diagnosis according to WHO (HbA1c≥6.5%). Significant differences in HbA1c levels between two groups were evident at the midpoint of the study and were further expanded at the final. Participants of the PGBR group were administered the same types of medicine as those in the WR group, indicating that a combination of medication treatment and PGBR diet was more effective than the single administration of anti-diabetic agents after a few years. The current data suggest that consuming PGBR in place of WR may be useful in modulating blood glucose in Vietnamese type 2 DM patients.

WR is the principle staple food consumed in Vietnam, where 66% of the energy intake was contributed by rice. As previously reported, the average BMI of newly diagnosed DM patients in Vietnam was approximately 23. High WR intake is considered a major cause of DM at low BMI in Asian populations, since WR has been classified as a high GI food. To produce WR, bran and germ of BR is eliminated through milling and refining processes, which dispossess WR of vital nutrients and phytonutrients, including dietary fiber, minerals, GABA, oryzanols, and fatty acids (11, 22-23). WR therefore is mainly composed of starchy endosperm and is rich in refined carbohydrates, which explains the high GI value. BR and PGBR, on the other hand, retain the dietary fiber-rich rice bran, which makes the most prominent distinction between them and WR. Compared to WR, BR and PGBR have lower GI values, and have been demonstrated to attenuate glycemic response (21-23, 35).

It is noteworthy that in the current study, dietary fiber intakes of the subjects at baseline were around 8 g/day, substantially lower than the recommended intake of 18-20 g/day by Vietnam's Ministry of Health (25). In line with previous papers, the present results revealed a marked difference in dietary fiber intake of the two study groups at final, with mean fiber intake of the PGBR group being significantly higher (10.9 g/day and 7.7 g/day in the PGBR and WR group, respectively). Dietary fiber has been known to be beneficial in regulating blood glucose and lipid concentrations (26-28). In the gastrointestinal tract, fiber passes through unabsorbed and unchanged. Fiber increases bulk of intestinal contents and viscosity of digestive solutions, blunting the diffusion of glucose to the intestinal walls, decelerating the processes of carbohydrate digestion as well as glucose absorption (27). As a consequence, sugar is absorbed into the bloodstream more slowly, and the following increment of the post-prandial blood glucose level is reduced in rapidity. This may be one of the chief physiological mechanisms correlated with GI values of foods, giving an explanation for the difference in GI classification among WR, BR and PGBR. Substitution of PGBR for WR was shown to significantly lower the threat of developing diabetes risk factors, and the credits were given to dietary fiber contents in PGBR bran (21-22, 24). Therefore, a daily addition of 3 g fiber in the diet of the PGBR group may be one of the foremost factors contributing to the HbA1c improvement found in this study.

Fiber may not be the sole factor explaining the results of the present study. GABA, γ-oryzanol and ASG are among various bioactive components of which concentrations were potentiated during the germination process of PGBR. GABA is the main inhibitory neurotransmitter produced by pancreatic β-cells and was demonstrated to induce stimulated insulin secretion (28). In PGBR, GABA concentration is 5-8 times higher than that of WR (21). Recent studies have shown that GABA improved hyperglycemia of diabetic mouse models and promoted β-cell proliferation (28-30). It has been reported that in grafted human islets, GABA activates a Ca^{2+}-dependent signaling pathway through $GABA_A$ receptor and $GABA_B$ receptor, a process in which activation of phosphatidylinositol 3-kinase (PI3K)/AKT signaling is involved. PI3K/AKT is known to be critical for the modulation of β-cell mass and acts in response to Glucagon-like peptide-1 (GLP-1) and glucose in rodents (31), which is in accord with findings by Purwana et al (30) in human islets. It can be presumed from GABA administration outcomes that the improvement in blood glucose concentration may be derived from beneficial impacts of GABA in fortifying human β-cell mass, increasing human insulin circulation, and decreasing glucagon levels. γ-oryzanol is another major bioactive components found in BR as well as PGBR. It is a compound of ferulic acid esters with phytosterols. Oryzanol (Orz) has been reported to ameliorate glucose intolerance and significantly reduced hypothalamic endoplasmic reticulum (ER) stress in rodents. Hypothalamic ER stress has been known as a factor in systemic glucose intolerance by inducing ER stress in the liver and adipose tissue, while an 8-week oral Orz treatment was confirmed to improve glucose metabolism in high fat diet fed mice (32). Preclinical findings have shown multiple effects of oryzanol and ferulic acid, including anti-oxidative (32-33), anti-hypoadiponectinemia and anti-hyperlipidemia (34-35). Furthermore, ASG contents found in PGBR bran was reported by Usuki et al to increase Insulin growth factor-1 (GF-1) generation in pancreatic β-cells of streptocizotocin-induced diabetic rats, which in turn remarkably reduced hyperglycemia (36). ASG was concurrently demonstrated to recover the streptocizotocin-induced decrease in activities of homocysteine-thiolactonase (HTase), an anti-oxidant enzyme whose activation is associated with decreased glycolysis in oxidative-stressed cells.

Until now, preclinical studies have evaluated effects of GABA, oryzanol, ASG and other PGBR components as individual bioactive compounds, at times with much higher doses compared to those available in PGBR. Purwana et al administrated 6-30 mg GABA per 1 mL drinking water on rodents (30), while GABA concentration in PGBR was 3-15 mg/100 g rice (21-22). Nevertheless, PGBR diet administrations have exhibited significant outcomes as reported by several previous papers. This suggests that the improvement in HbA1c found in this study were elicited from the synergy among various bioactive compounds in combination with dietary fiber when consumed as a whole food (PGBR), rather than the impacts of any individual components (37-38). It is likely that the overall synchronous activities of these compounds, or "food energy" (39), were what optimized the comprehensive effects, as evidenced by the noteworthy outcomes of PGBR diet.

As opposed to fasting blood glucose concentration level which is afflicted by daily fluctuations, HbA1c is a more reliable measure for clinical assessment of glycemic control in DM patients (39). HbA1c is formed by non-enzymatic attachment of glucose to the N-terminal valine of the hemoglobin β-chain (40). The concentration of HbA_{1c} depends on both the blood glucose concentration and the life span of the red blood cells (RBCs). As human RBCs live in blood circulation for roughly 100-120 days, HbA_{1c} level reflects the glucose concentration accumulated over a course of 8–12 weeks (41). Accordingly, this study was designed to last 4 months to accurately elucidate the effects of PGBR on HbA1c levels of the subjects.

Preliminary results in physical activity intensity (represented by footstep counts) of both groups exhibited particular difference (p=0.026). However, over the 4-month intervention, both groups showed no sign of any within-group changes in physical activity intensity (p=0.206 and p=0.713 in PGBR group and WR group, respectively). Despite the fact that marked declines in body fat percentage were observed, no significant difference was found in body weight among both groups, in contrast with the results of a previous study (24). As type 2 DM patients, all subjects strictly follow their individualized diet, as reflected in the similar energy and nutrient intake at baseline and final in both groups. It is widely known that energy balance is what control body weight. Therefore, the stability in body weight observed in our data can be explained by the well balanced energy intake and constant physical activity intensity of study participants. A study with similar design in younger, non-diabetic subjects has been planned to corroborate the previous body weight results.

CONCLUSION

The present study suggests that substitution of WR with PGBR can improve HbA1c levels of Vietnamese type 2 DM patients who have been under long-term oral diabetes treatment. In Vietnam where rice contributes to 67% of the daily caloric intake, the escalation of T2DM cases could be regulated owing in large part to the widespread use of PGBR.

ACKNOWLEDGEMENT

The authors are grateful to all the study participants and workers of Nam Dinh Endocrinology Hospital. This study was supported by FANCL Research Institute of FANCL Corp., Japan, Vietnam's National Institute of Nutrition, US-Japan Medical Science Program, and Asian Nutrition and Food Culture Research Center, Jumonji University, Japan.

REFERENCES

1) World Health Organization. The top 10 causes of death. 2013.
2) International Diabetes Federation. IDF Diabetes Atlas. 2013.
3) Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. 2010. *Diabetes Res Clin Pract* 87:4-14.
4) WHO expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. 2004. *Lancet* 363:157-163.
5) Yoon KH, Lee JH, Kim JW, Cho JH, Choi YH, Ko SH, Zimmet P, Son HY. Epidemic obesity and type 2 diabetes in Asia. 2006. *Lancet* 368:1681-1688.
6) Lin YC, Yen LL, Chen SY, Kao MD, Tzeng MS, Huang PC, Pan WH. Prevalence of overweight and obesity and its associated factors: findings from National Nutrition and Health Survey in Taiwan, 1993-1996. 2003. *Preventive Medicine* 37:233-241.
7) Le Nguyen TD, Tran TM, Kusama K, Ichikawa Y, Nguyen TK, Yamamoto S. Vietnamese type 2 diabetic subjects with normal BMI but high body fat. 2003. *Diabetes Care* 26:1946-1947.
8) Son LNTD, Hanh TT, Kusama K, Kunii D, Sakai T, Hung NT, Yamamoto S. Anthropometric characteristics, dietary patterns and risk of type 2 diabetes mellitus in Vietnam. 2005. *J Am Coll Nutr* 24:229-234.
9) Yamamoto S, Le Nguyen TD, Hsu TH, Huang KC, Hung NV, Wong Y, Huang PC. Vietnamese may develop type 2 diabetes with smaller increases in body mass index and waist circumference than those in Taiwan. 2013. *Int J Diabetol Vas Dis Res* 1:1-5.
10) Hu EA, Pan A, Malik V, Sun Q. White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. 2012. *BMJ* 344:e1454.
11) Sun Q, Spiegelman D, van Dam RM, Holmes MD, Malik VS, Willett WC, Hu HB. White rice, brown rice, and risk of type 2 diabetes in US men and women. 2010. *Arch Intern Med* 170:961-969.
12) Jenkins DJA, Kendall CWC, Augustin LSA, Vuksan V. High-Complex Carbohydrate or Lente Carbohydrate Foods? 2002. *Am. J. Med* 113(9B):30S-37S.
13) Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. 2004. *Am J Clin Nutr* 80(2):348-356.
14) Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, Mitchell P, Brand-Miller JC. Glycemic index, glycemic load, and chronic disease risk--a meta-analysis of observational studies. 2008. *Am J Clin Nutr* 87(3):627-637.
15) Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. 2008. *Diabetes Care* 31(12):2281-2283.
16) Nanri A, Mizoue T, Noda M, Takahashi Y, Kato M, Inoue M, Tsugane S, Japan Public Health Center-based Prospective Study Group. Rice intake and type 2 diabetes in Japanese men and women: the Japan Public Health Center-based Prospective Study. 2010. *Am J Clin Nutr* 92(6):1468-1477.
17) National Institute of Nutrition. 2010. National Nutrition Survey 2009-2010. Hanoi Medical Publisher, Hanoi (in Vietnamese).
18) Riccardi G, Rivellese AA, Giacco R. Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. 2008. *Am J Clin Nutr* 87(1):269S-274S.
19) Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu S. Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular

disease, and weight gain. 2012. *J Nutr* 142:1304-1313.
20) J. Brand-Miller, S. Hayne, P. Petocz, S. Colagiuri. Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized controlled trials. 2003. *Diabetes Care* 26(8):2261-2267.
21) Ito Y, Mizukuchi A, Kise M, Aoto H, Yamamoto S, Yoshihara R, Yokoyama J. Post-prandial blood glucose and insulin responses to pre-germinated brown rice in healthy subjects. 2005. *J Med Invest* 52(3-4):159-164.
22) Hsu TF, Kise M, Wang MF, Ito Y, Yang MD, Aoto H, Yoshihara R, Yokohama J, Kunii D, Yamamoto S. Effects of pre-germinated brown rice on blood glucose and lipid levels in free-living patients with impaired fasting glucose or type 2 diabetes. 2008. *J Nutr Sci Vitaminol* 54(2):163-168.
23) Patil S.B., Khan M.K. Germinated brown rice as a value added rice product: A review. 2011. *J Food Sci Technol* 48:661-667.
24) Nhung BT, Hop LT, Huy ND, Binh TQ, Lam NT, Tuyen LD, Anh NDV, Linh VA, Aoto H, Okuhara Y, Ito Y, Yamamoto S, Kise M. Pre-germinated Brown Rice Reduced Both Blood Glucose Concentration and Body Weight in Vietnamese Women with Impaired Glucose Tolerance. 2014. *J Nutr Sci Vitaminol* 60:183-187.
25) Vietnam Ministry of Health. Vietnamese Dietary Reference Intake, 2007. p 39-40. Hanoi Medical Publisher, Hanoi (in Vietnamese).
26) Riccardi G, Rivellse AA. Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. 1991. *Diabetes Care* 14(12):1115-1125.
27) Ou S, Kwok K, Li Y, Fu L. In Vitro Study of Possible Role of Dietary Fiber in Lowering Postprandial Serum Glucose. 2001. *J Agric Food Chem* 49(2):1026-1029.
28) Soltani N, Qiu H, Aleksic M, Glinka Y, Zhao F, Liu R, Li Y, Zhang N, Chakrabarti R, Ng T, Jin T. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. 2011. *Proc Natl Acad Sci USA* 108(28):11692–11697.
29) Gomez R, Asnis N, Tannhauser SL, Barros HMT. GABA agonists differentially modify blood glucose levels of diabetic rats. 1999. *Japanese Journal of Pharmacology* 80(4):327–331.
30) Purwana , Zheng J, Li X, Deurloo M, Son DO, Zhang Z, Liang C, Shen E, Tadkase A, Feng ZP, Li Y, Hasilo C, Paraskevas S, Bortell R, Greiner DL, Atkinson M, Prud'homme GJ, Wang Q. GABA Promotes Human β-Cell Proliferation and Modulates Glucose Homeostasis. 2014. *Diabetes* 63(12):4197-4205.
31) Elghazi L, Rachidi L, Weiss AJ, Cras-Neneur C, Bernal-Mizrachi E. Regulation of beta-cell mass and function by the Akt/protein kinase B signalling pathway. 2007. *Diabetes Obes Metab* 9 (Suppl. 2):147–157.
32) Kozuka C, Yabiku K, Sunagawa S, Ueda R, Taira SI, Ohshiro H, Ikema T, Yamakawa K, Higa M, Tanaka H, Takayama C, Matsushita M, Oyadomari S, Shimabukuro M, Masuzaki H. Brown Rice and Its Component, g-Oryzanol, Attenuate the Preference for High-Fat Diet by Decreasing Hypothalamic Endoplasmic Reticulum Stress in Mice. 2012. *Diabetes* 61(12):3084–3093.
33) Lerma-Garcia MJ, Herrero-Martinez JM, Simo-Alfonso EF, Mendonca CRB, Ramis-Ramos G. Composition, industrial processing and applications of rice bran gamma-oryzanol. 2009. *Food Chem* 115:389–404.
34) Nagasaki R, Yamsaki T, Uchida A, Ohara K, Ushio H. γ-Oryzanol recovers mouse hypoadiponectinemia induced by animal fat ingestion. 2011. *Phytomedicine* 18(8-9):669–671.
35) Ghatak S, Panchal S. Gamma-oryzanol – A multi-purpose steryl ferulate. 2011. *Current Nutrition & Food Science* 7:10–20.
36) Usuki S, Tsai YY, Morikawa K, Nonaka S, Okuhara Y, Kise M, Yu RK. IGF-1 induction by acylated steryl β-glucosides found in a pre-germinated brown rice diet reduces oxidative stress in streptozotocin-induced diabetes. 2011. *PLoS ONE* 6 (12):e28693.
37) Jacobs DR Jr., Steffen LM. Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. 2003. *Am J Clin Nutr* 78(3 Suppl):508S-513S.
38) Imam MU, Azmi NH, Bhanger MI, Ismail N, Ismail M. Antidiabetic Properties of Germinated Brown Rice: A Systematic Review. 2012. *Evid Based Complement Alternat Med*: 816501.
39) World Health Organization. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus, Abbreviated Report of a WHO Consultation. 2011:1-25.
40) Sacks DB. Carbohydrates. *In*: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz textbook of clinical chemistry and molecular diagnostics, 2006. p 837–902. St. Louis: Elsevier Saunders.
41) Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, Sacks DB. Test of Glycemia in Diabetes. 2004. *Diabetes Care* 27(7):1761-1773.Hayes B, Lee K. Head size and growth in the very preterm infant: a literature review. Res Reports Neonatol 5:1-7. 201

RESEACH NOTE

The Effect of A School-based Nutrition Education Program on Japanese Elementary School Children's Nutrition Knowledge and The Revaluation after One and A Half Year.

Mai Matsumoto[1, 2], Ikumi Goto[2], Ayumi Masumoto[3],
Azusa Sakamoto[2], Aki Saito[1], Shinji Ikemoto[2] *

[1] *Department of Nutritional Epidemiology and Shokuiku, National Institutes of Bioinnovation, Health and Nutrition., Shinjuku-ku, Tokyo, Japan*
[2] *Department of Human Nutrition, SEITOKU University., Matsudo-shi, Chiba, Japan*
[3] *Saitama city., Saitama-shi, Saitama, Japan*

(received November 30, 2018)

ABSTRACT *Background and purpose*. Nutrition knowledge is one of the factors influencing on diet. Children's nutrition knowledge was reported to increase after they received nutrition education classes. However, there were few reports about the continuation of effect after the nutrition education on children's nutrition knowledge. This study aim was to evaluate the continued efficacy of a school-based nutrition education program on children's nutrition knowledge specializing in food grouping for one and a half year among elementary school children using pre-post survey. *Methods.* Participants were Japanese elementary school students in second grade (n=53). Two-time nutrition education lectures on the "three-color food groups" held as part of a school-based seven-month dietary education program. The changes in children's nutrition knowledge scores by the nutrition education program of "Three-color food groups" evaluated by a questionnaire about "Three-color food groups" including two sections of "Color" and "Function" from Pre-intervention to After-class (immediately following lectures), Post-intervention (immediately after all programs for seven months) and After-a-year-intervention (one year following the intervention). The Friedman test and Scheffe's method were used to those analyses. *Results.* Both "Color" and "Function" scores were higher at After-class, Post-intervention, and After-a-year-intervention than Pre-intervention ($P < 0.001$ for all), while the "Function" score declined at Post-intervention and A-year-after-intervention compared to After-class. *Conclusion.* A single program specialized in "Color" of "Three-color food groups" may improve knowledge and continue its effect among children aged 7-8 years, although repeated familiarization may be needed until they understand difficult or unfamiliar concepts or words such as "Function".
Keywords: School-based nutrition education, Nutrition knowledge, Elementary schools, Children, Food groups

INTRODUCTION

School age is crucial years for normal physical and mental development, and the diet and eating behaviour that develop during these years tend to persist throughout life (1) is a period in which food preference and eating habits are formed. Childhood is a time of greater openness to modification of food choice (2), therefore, the eating habits and behaviors formed in childhood may be difficult to change later in life (2-4).

Children's diet is influenced by various factors, such as their own knowledge (5), their parents' nutrition knowledge (6,7), and home food environment (6,8). In particular, nutrition knowledge is necessary for making healthier food choices (7,9-11), and it has been found to predict dietary quality (11). Moreover, the knowledge about food and its health benefits that children gain in childhood can influence their dietary choices and preferences later in life (5,12). For example, a Japanese study reported that elementary school students with higher nutrition knowledge consumed more vegetables than students with lower nutrition knowledge (13). Thus, it might be necessary to obtain nutrition knowledge during childhood in order to benefit health later in life.

A number of nutrition-education programs are designed to improve nutrition knowledge, with the aim of supporting sound dietary intake within the community or a specific target population (6,8). Nutrition-education programs that present positive dietary messages can potentially improve dietary behavior and increase nutrition knowledge in children (6). In fact, several studies about nutrition education have been reported. A review reported that school-based, nutrition education programs can lead to moderate increases in vegetable consumption

*To whom correspondence should be addressed: S Ikemoto: ikemoto@seitoku.ac.jp

among children (14). A UK study reported that nutrition education improved 5-7 year-olds' nutrition knowledge (15). Several studies have reported that gardening improved preference and consumption of fruits and vegetables in younger generations (16-18). In particular, school-based nutrition education can be delivered in a way that is easily understood by children, and teach the skills and knowledge required to improve or strengthen healthy eating habits (19). For example, it is reported that school food policies may encourage healthy eating and discourage consumption of unhealthy foods and sweetened drinks (20,21). The previous study showed that nutrition education on food groups for young Japanese children improved their nutrition knowledge (22). Another study reported that nutrition education on healthy food was effective in helping young children increase nutrition knowledge (23). That is to say school-based nutrition education has impacted improving children's nutrition knowledge and food behavior.

The ultimately goal is to increase nutrition knowledge and positive behavior change, which can have a beneficial effect on the long-term health of children enrolled in any such nutrition-education programs (15). However, there are few studies on the effect of the continuousness of these programs. It is important to investigate what undermines the continuousness of such programs in order to implement effective interventions. Therefore, the aim of this study was to assess the continued efficacy of a school-based nutrition education program on 7-8 year old elementary school children's nutrition knowledge for one and a half years.

METHODS

Study design. This before-after trial evaluated a school-based nutrition education program on nutrition knowledge of "Three-color food groups" for second grade students (7-8-year-olds) in a public elementary school in Saitama city of Japan. The nutrition-education program was conducted as part of a school-based, seven-month, dietary education program.

Pre-intervention assessment of nutrition knowledge was conducted using a questionnaire for participants in May 2012 (Pre-intervention). The school-based, dietary education program was conducted for seven months after the Pre-intervention assessment. This program included a series of nutrition-education lectures on the "Three-color food groups", which was conducted twice in October and November of 2012. The nutrition knowledge assessment using the same questionnaire as Pre-intervention was conducted after the series of lectures (After-class), immediately after all programs for seven months (Post-intervention), and one year after the Post-intervention in February 2014 (After-a-year-intervention).

The present study was granted ethical approval by the ethics committee of Seitoku University in accordance with the "Helsinki Declaration: Ethical Principles for Research Involving Human Subjects" (H24U003).

Subjects. The Figure 1 shows an overview of the number of participants in the present study. Seventy-nine second grade students of an elementary school in Saitama city in Japan were recruited. There were no exclusion criteria. A total of 59 students completed all interventions, including four nutrition-knowledge assessments and the nutrition-education program on "Three-color food groups". Finally, 53 (28 male and 25 female) students completed all programs.

The nature of the study was explained to the school principal and homeroom teachers by the

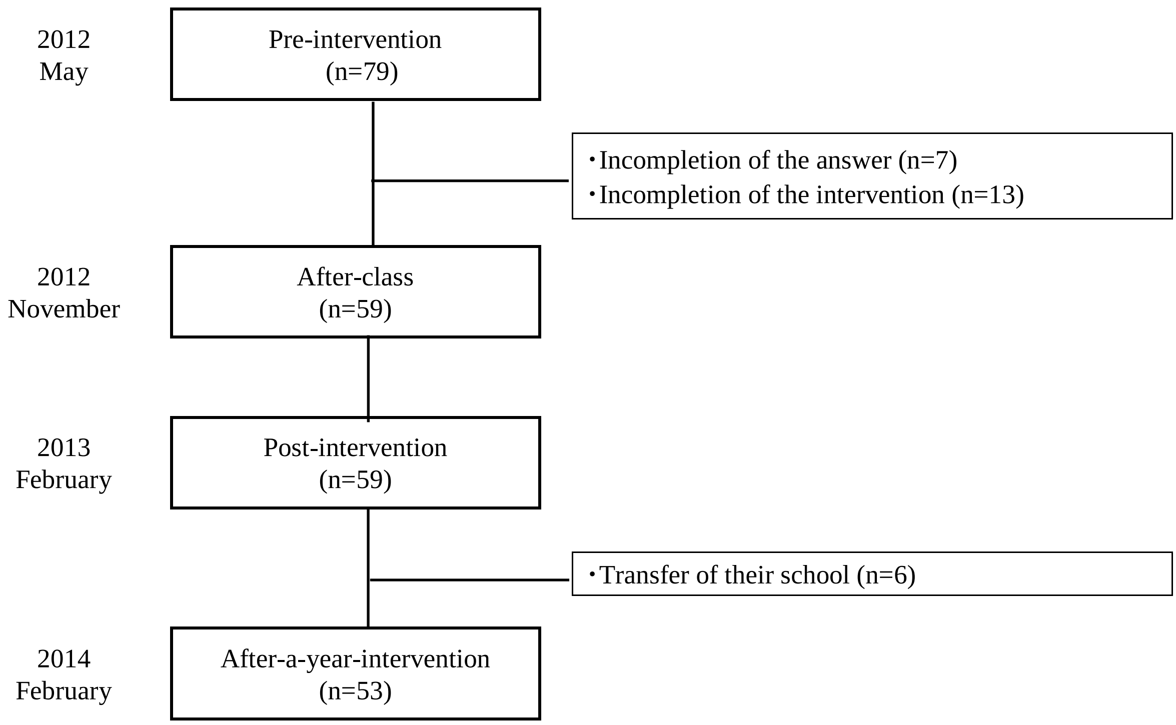

Figure 1. Overview of the number of participants contained in this study

researcher and distributed by mail to the parents of children who participated in the study. Written consent forms were obtained from parents before the commencement of the study.

Intervention program. The school-based, seven-month dietary education program was composed of lectures on six topics: "Breakfast and life-rhythm", "Healthy snacking behavior", "Food poisoning", "Three-color food groups", "Eating manner" and "Seasonal foods". The program was developed for the school by teachers and the researcher, based on the principal components of "Basic Law on Shokuiku" (24), "Basic Program for Shokuiku Promotion" (25) and "Elementary school curriculum guideline" (26). The lecture on the "Three-color food groups" was conducted twice, while the others were conducted once. "Three-color food groups" is a classification system recommended to be taught in home economics at Japanese elementary schools for students to easily understand well-balanced dietary intake (26). According to the system, food can be classified into three color groups, including "Red", "Green," and "Yellow" by the function of the dominant nutrients in each food. The "Red" food group includes meat, fish, soybeans, and eggs, which are all rich in protein, and work to build the blood and body. The "Green" food group includes vegetables and fruits, which are rich in vitamins and minerals, and work to keep the body healthy. The "Yellow" food group includes rice, bread, noodles, potatoes, and oil, which are rich in carbohydrates or fats, and work as the source of the body's energy. The example of lecture about "Three-color food groups" is shown in Figure 2. The lectures included the definition and classification of food groups based on "Three-color food groups" system so the children could understand the differences of nutritional functions in the various food groups and their combination to achieve a well-balanced diet. The other lectures in the program provided information on basic dietary behaviors. For example, the lectures on "Breakfast and life-rhythm" and "Healthy snacking behavior" comprised the roles of eating breakfast and snaking in children's lifestyle. The lecture on "Food poisoning" provided information on preventing food poisoning and related behaviors. In the lecture on "Eating manner", children were taught a good table manner, such as how to use chopsticks. The lecture on "Seasonal food" comprised the best season for eating vegetables and fruits.

The program was completed by a participatory approach, using a black board and card games aimed at building students' nutrition knowledge, which amounted to seven interventions in total. Each lecture was given for a 30-minute period and were conducted by the same registered dietitians. Since the duration of a lecture at elementary schools in Japan is 45 minutes, a 30-minute lecture was deemed suitable for the study participants.

Nutrition Knowledge Questionnaire on the "Three-color Food Groups" for Young Children. A questionnaire was developed to assess students' nutrition knowledge focusing on the "Three-color food groups" and based on the contents of the nutrition-education program. The contents of the questionnaire are shown in Table 1.

The questionnaire consisted of six items asking students of their nutrition knowledge of "Three-color food groups". It took approximately 5 to 10 minutes to complete the questionnaire, which is a suitable length of time for children of the participants' age group (27). This questionnaire is a multiple-choice test in which participants were given a choice of four answers, plus an option of "I don't know". Before the first assessment, the components of the questionnaire were reviewed by the school principal, teachers (n=3), and registered dietitians (n=2) to ensure its appropriateness. Internal consistency of the questionnaire was calculated by using Cronbach's α was 0.603 among 25 children, age 6 in the pilot study (data not shown). The questionnaire was conducted by an anonymous self-completed method in each class. A researcher read questions aloud to each class because it was reported that it was preferable for the proctor to read questions aloud in order to minimize the impact of reading levels on results (27).

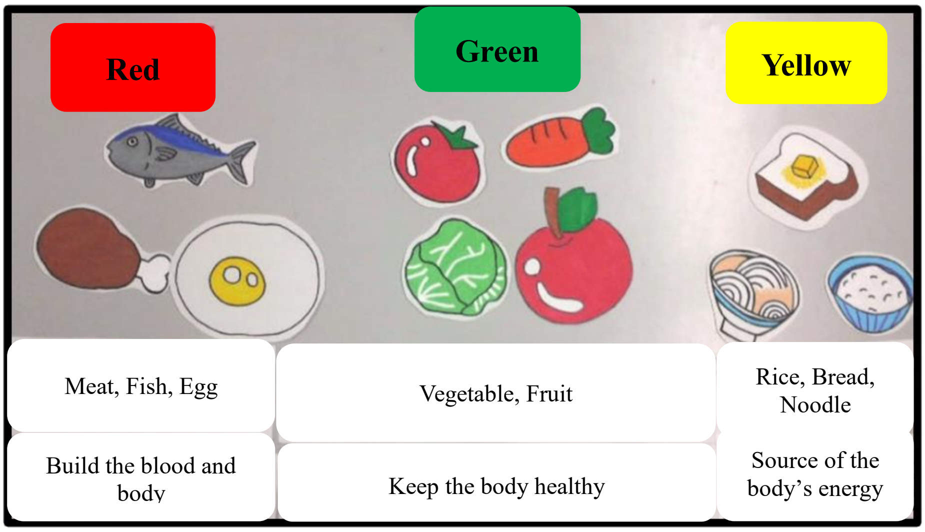

Figure 2. The content of lecture about "Three-color food groups" system

Table 1. Content of the Questionnaire for Children

Category	Sub-category	Question number	Content
Three-color food groups	Color	No.1	"Three-color food groups" Which food is included in the "red group"?
		No.2	"Three-color food groups" Which food is included in the "green group"?
		No.3	"Three-color food groups" Which food is included in the "yellow group"?
	Function	No.4	"Three-color food groups" Which food is the source of energy?
		No.5	"Three-color food groups" Which food is the source of blood or muscle?
		No.6	"Three-color food groups" Which food helps maintain proper bodily functions?

Table 2. Anthropometric Data for Students who Participated in Study

	All (n=53)		Male (n=28)		Female (n=25)		p
	Median value	(IQR)	Median value	(IQR)	Median value	(IQR)	
Body height (cm)	120.0	(118.0-125.0)	120.0	(118.5-125.5)	121.0	(118.0-123.0)	0.713
Body weight (kg)	22.5	(20.0-25.0)	22.0	(20.5-25.0)	22.8	(20.0-25.0)	0.781
Body mass index (kg/m^2)	15.3	(14.4-16.9)	15.3	(14.1-17.5)	15.1	(14.5-16.8)	0.915

The p values were calculated by Mann-Whitney test.

Other Variables. Body weight and body height were obtained by school health check data. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of body height (in meters).

Statistical analysis. All statistical analyses were performed using the IBM SPSS statistics software package (version 22.0, SPSS Inc., Chicago, IL, USA). The responses of the nutrition-knowledge questionnaire were converted to scores of '1' and '0' for correct and incorrect answers, respectively. The answer of "I don't know" was scored as '0'. Scores were calculated by summing the total number of correct answers. A higher score reflected a higher knowledge level. The difference of body height, weight, and BMI between male and female students participating in the study was compared by the Mann-Whitney test. The effect of the continuousness of the nutrition-education program was assessed by comparison of nutrition knowledge of the "three-color food groups" for a total of four interventions, including Pre-intervention, After-class, Post-intervention, and A-year-after-intervention by the Friedman test, using Scheffe's method to compare the score between each intervention.

RESULTS

Demographic characteristics of the study participants are presented in Table 2. Median body height, weight, and BMI were not significantly different between male and female students.

Table 3 shows the scores of the "Three-color food groups" knowledge questionnaire at Pre-intervention, After-class, Post-intervention, and After-a-year-intervention. The knowledge score of "Color" was improved at After-class, Post-intervention, and A-year-after-intervention from Pre-intervention, respectively ($P < 0.001$). The knowledge score of "Function" of each color was improved at After-class, in comparison to Pre-intervention ($P < 0.001$). The score of "Function" declined at Post-intervention and A-year-after-intervention from After-class, while these scores were significantly higher compared to the score of Pre-intervention. The difference in sex did not affect these results (data not shown).

DISCUSSION

In this study, we examined whether the effect of a school-based nutrition education program on the nutrition knowledge of 7-8 year-olds continued for

Table 3. Score of Knowledge of Questionnaire to Assess Effect of Nutrition Education of the "Three-color food groups" (n=53)

	Pre-intervention		After-class		Post-intervention		A-year-after-intervention		p
	Median value	(IQR)	Median value	(IQR)	Median value	(IQR)	Median value	(IQR)	
"Color"	1.0	(1.0-2.0)^a	3.0	(3.0-3.0)^b	3.0	(3.0-3.0)^b	3.0	(3.0-3.0)^b	< 0.001
"Function"	1.0	(1.0-2.0)^a	3.0	(2.0-3.0)^b	2.0	(2.0-3.0)^c	2.0	(2.0-2.0)^c	< 0.001

IQR, interquartile range.
The p values were calculated by Friedman test.
$^{a\,b\,c}$ There is significant difference between different alphabet in the same row using by Scheffe's method to compare the score between each intervention

one and a half years. To the best of our knowledge, the present study is the first study to examine the continued efficacy of a nutrition-education program on children's nutrition knowledge for over a year. Our findings suggest that that the nutrition education specialized in "Color" of "Three-color continued has led to a one and a half year effect among 7-8-year-old children.

The effect of school-based nutrition education programs have been examined in various settings. For example, Connell, et al. and Resnicow, et al. reported that the evaluation of school health education improved healthy food behavior for elementary school students (28,29). In addition, the effect of school-based nutrition education specializing in nutrition knowledge has also been reported. Bargen reported that the evaluation of school health education showed that 10 to 15 hours were required to expect "large" effects in a program about specific knowledge (30). Morgan et al. found that a three-hour, school-based nutrition education program was a promising tool to improve 11-12-year-olds' nutrition knowledge and food preferences (8). The results of the previous studies were similar to our results, which showed that a nutrition-education program improved 7-8-year-olds' nutrition knowledge. Our participants obtained low scores (median 2 points out of 6) at the baseline assessment (Pre-intervention), while they obtained median 5-6 points after the intervention. A previous study showed that 4-6-year-olds did not have considerable knowledge on "Three-color food groups" used for the intervention (31). Together with this result, our participants were likely to obtain the knowledge in question from the intervention program, which indicated that nutrition-education programs focusing on nutrition knowledge may have an effect in improving and establishing nutrition knowledge for 7-8-year-olds.

For nutrition education, our study focused on the food grouping and nutrient functions using "Three-color food groups" system, both in education and assessment of nutrition knowledge. We selected this classification system because it has been employed for children to easily understand a balanced diet and is the tool to teach children food grouping in Japanese elementary schools. Previous studies comprised nutrition-knowledge questionnaires for young children, including the concept of food grouping in the view of nutrient function or healthy food intake (32,33), which made us assess the knowledge similarly. In this study, the assessment was conducted from two viewpoints: "Color" and "Function", which represented food grouping and nutrient function, respectively. A number of differences were observed between the results. Recognition of "Color" was maintained for an extended time after the improvement realized through the intervention. Therefore, a single nutrition education about "Color" may be appropriate for children of this age. However, although recognition of "Function" of each color improved in the After-class phase, the score slightly decreased following that (Post-intervention). A previous study using "Three-color food groups" reported that recognition of "Color" was significantly improved after an intervention while recognition of "Function" did not significantly improve among young Japanese children (22). Based on these findings in our results, it might be difficult for children to fully acquire knowledge on nutritional function. One possible reason for this could be familiarization after the intervention. Most Japanese elementary schools provide parents with monthly school-lunch program letters, which usually include information to classify the foods used in the lunch menu to the "Three-color food groups". After the children received nutrition education on "Three-color food groups", it is possible that consciousness of the monthly school-lunch program letter led to the foundation of the knowledge. On the contrary, it might be too difficult for the children to understand the definition of nutritional function, as the score declined after an extended period. In fact, nutrition education using "Three-color food groups" is mostly employed targeting fifth grade (10-11-year-olds) in Japan (34). Our subjects were much younger than the targeted children and it is possible that the words used for the functions were too complicated to remember. Birch et al. reported that five to six times more education were needed until children became familiar with vegetables or vegetable names that were new to them in 1987 (35). In addition, a study about children's nutrition knowledge showed that they had poor nutrition knowledge about food function and nutrients contained in food (32, 36). Thus, it may take a longer period of time for children to understand new or difficult words, particularly words that are unfamiliar to them. A follow-up strategy may be needed to maintain the nutritional knowledge such as "Function" gained in a nutrition-education program. Therefore, further study will be needed to resolve this question. Additionally, our results showed the improvement or continuance of nutrition knowledge did not significantly differ according to sex (data not shown). These results were similar to a previous study among 5-7-year-old children (15). Thus, the differences by sex in children of our participants' ages may have little influence in gaining nutrition knowledge.

Several limitations of the present study warrant highlighting. First, the number of subjects was small (n=53). However, several studies of nutrition education for children were conducted for a similar number of subjects (37). In addition, participants in this study were limited to children belonging to a public elementary school in Saitama city in Japan, not random samples from the general population. The participants were therefore likely not representative of Japanese children. Second, before-after trials without a control group was used in the present study. However, there are few studies on the effects of nutrition education on young children in Japan, and the effects of improvement and its continuousness for nutrition education were not found. It is said that sufficient information is needed to complete a randomized-controlled trial and the effects should be investigated without a control group before such a trial is completed (38). Thus, it is important that the effect of improvement of continuousness on nutrition education was investigated without a control group, such as the previous study (37) the present study completed. Third, the Cronbach's α value of the questionnaire was lower in comparison to the reference value of 0.7 (39). Cronbach's α coefficient is estimated to be higher when the number of items increases (40). The low α value obtained in this questionnaire may be attributed to the small item numbers. Fourth, in the present study, the same questionnaire was used for each nutrition-knowledge assessment, and it was possible that children remembered the correct answers of the questionnaire. However, according to previous studies reporting reproducibility of questionnaires of nutrition knowledge targeting children, our questionnaires were conducted 2-4 weeks apart because children were likely to forget the correct answers during that time (27,41). Our examination was conducted within at least three-month intervals; as such, we believed that using the same questionnaire did not influence the current results. Lastly, we did not ask participants about household income, family environment, and their parents' nutrition knowledge. It was reported that household income and family environment affected nutrition knowledge (42,43), and parents' knowledge made an impact on children's knowledge (41). Additional studies that include those factors that influence children's nutrition knowledge should be conducted.

CONCLUSION

In conclusion, a school-based nutrition education program focused on the "Three-color food groups" improved nutrition knowledge among 7-8-year-old Japanese children. Moreover, the obtained knowledge specialized in "Color" was likely to remain for one and a half years. Therefore, a single nutrition education about "Three-color food groups" specialized in "Color" may be appropriate for children of this age. In order for children to gain knowledge, repeated familiarization may be needed until they understand difficult or unfamiliar concepts or words such as "Function". Future studies are needed to determine the contents and components of effective nutrition-education programs to improve both nutrition knowledge and dietary behavior of young children further.

ACKNOWLEDGEMENT

The authors would like to express their deepest appreciation to all the study participants: school principals, teachers, students and their parents. The authors have no potential conflicts of interest to disclose.

REFERENCES

1) Wahl R. Nutrition in the adolescent. Pediatr Ann 28:107-111. 1999.
2) Wardle J. Parental influences on children's diets. Proc Nutr Soc 54:747-758. 1995.
3) Campbell KJ, Hesketh KD. Strategies which aim to positively impact on weight, physical activity, diet and sedentary behaviours in children from zero to five years. A systematic review of the literature. Obes Rev 8: 327-338. 2007.
4) Kelder SH, Perry CL, Klepp KI, Lytle LL. Longitudinal tracking of adolescent smoking, physical activity, and food choice behaviors. Am J Public Health 84: 1121-1126. 1994.
5) Backman DR, Haddad EH, Lee JW, Johnston PK, Hodgkin GE. Psychosocial predictors of healthful dietary behavior in adolescents. J Nutr Educ Behav 34: 184-192. 2002.
6) Powers AR, Struempler BJ, Guarino A, Parmer SM. Effects of a nutrition education program on the dietary behavior and nutrition knowledge of second-grade and third-grade students. J Sch Health 75: 129-133. 2005.
7) Worsley A. Nutrition knowledge and food consumption: can nutrition knowledge change food behaviour? Asia Pac J Clin Nutr 11 Suppl 3: S579-585. 2002.
8) Morgan PJ, Warren JM, Lubans DR, Saunders KL, Quick GI, Collins CE. The impact of nutrition education with and without a school garden on knowledge, vegetable intake and preferences and quality of school life among primary-school students. Public Health Nutr 13:1931-1940. 2010.
9) Gibson EL, Wardle J, Watts CJ. Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. Appetite 31: 205-228. 1998.
10) Wardle J, Parmenter K, Waller J. Nutrition knowledge and food intake. Appetite 34: 269-275. 2000.
11) Axelson ML, Federline TL, Brinberg D. A meta-analysis of food- and nutrition-related research. Journal of Nutrition Education. 17: 51-54. 1985.
12) Branen L, Fletcher J. Comparison of College Students' Current Eating Habits and Recollections of Their Childhood Food Practices. J Nutr Educ 31: 304-310. 1999.
13) Asakura K, Todoriki H, Sasaki S. Relationship between nutrition knowledge and dietary intake among primary school children in Japan: Combined effect of children's and their guardians' knowledge. J Epidemiol. 27: 483-491. 2017.
14) Howerton MW, Bell BS, Dodd KW, Berrigan D, Stolzenberg-Solomon R, Nebeling L. School-based nutrition programs produced a moderate increase in fruit and vegetable consumption: meta and pooling analyses from 7 studies. J Nutr Educ Behav 39: 186-196. 2007.
15) Warren JM, Henry CJ, Lightowler HJ, Bradshaw SM, Perwaiz S. Evaluation of a pilot school programme aimed at the prevention of obesity in children. Health Promot Int. 18: 287-296. 2003.
16) Heim S, Stang J, Ireland M. A garden pilot project enhances fruit and vegetable consumption among children. J Am Diet Assoc 109: 1220-1226. 2009.
17) McAleese JD, Rankin LL. Garden-based nutrition education affects fruit and vegetable consumption in sixth-grade adolescents. J Am Diet Assoc 107: 662-665. 2007.
18) Hermann JR, Parker SP, Brown BJ, Siewe YJ, Denney BA, Walker SJ. After-school gardening improves children's reported vegetable intake and physical activity. J Nutr Educ Behav 38: 201-202. 2006.
19) Perez-Rodrigo C, Aranceta J. School-based nutrition education: lessons learned and new perspectives. Public Health Nutr 4: 131-139. 2001.
20) Bere E, Hilsen M, Klepp KI. Effect of the nationwide free school fruit scheme in Norway. Br J Nutr 104: 589-594. 2010.
21) Cullen KW, Watson K, Zakeri I. Improvements in middle school student dietary intake after implementation of the Texas Public School Nutrition Policy. Am J Public Health 98: 111-117. 2008.
22) Takao Y, Adachi N, Matsumoto M, Ikemoto S. The Efficiency of Intervention on Diet and

Health Education to Nursery School Students and Their Guardians. J Jap Diet Associatio 53: 246-251. 2010. (in Japanese)
23) Katz DL, Katz CS, Treu JA, Reynolds J, Njike V, Walker J, Smith E, Michael J. Teaching healthful food choices to elementary school students and their parents: the Nutrition Detectives program. J Sch Health 81: 21-28. 2011.
24) Cabinet Office, Government of Japan. Basic Law on Shokuiku, vol. 63. 2005. (in Japanese).
25) Cabinet Office, Government of Japan. Basic Program for Shokuiku Promotion. 2011. (in Japanese).
26) Ministry of Education, Culture, Sports, Science and Technology. JAPAN. Elementary school curriculum guideline. 2009. (in Japanese)
27) Gower JR, Moyer-Mileur LJ, Wilkinson RD, Slater H, Jordan KC. Validity and reliability of a nutrition knowledge survey for assessment in elementary school children. J Am Diet Assoc 110: 452-456. 2010.
28) Connell DB, Turner RR, Mason EF. Summary of findings of the School Health Education Evaluation: health promotion effectiveness, implementation, and costs. J Sch Health 55: 316-321. 1985.
29) Resnicow K, Cohn L, Reinhardt J, Cross D, Futterman R, Kirschner E, Wynder EL, Allegrante JP. A three-year evaluation of the know your body program in inner-city schoolchildren. Health Educ Q 19: 463-480. 1992.
30) Bergen D. Teaching Strategies: Authentic Performance Assessments. Childhood Education 70: 99-102. 1993.
31) Matsumoto M, Ikemoto S. The Necessary Items for Inclusion in a Questionnaire for Assessing the Nutrition Knowledge of Young Japanese Children. J Nutr Sci Vitaminol (Tokyo) 63: 8-14. 2017.
32) Lin W, Yang HC, Hang CM, Pan WH. Nutrition knowledge, attitude, and behavior of Taiwanese elementary school children. Asia Pac J Clin Nutr 16 Suppl 2: 534-546. 2007.
33) Grosso G, Mistretta A, Turconi G, Cena H, Roggi C, Galvano F. 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 Nutr 16: 1827-1836. 2013.
34) Kojima Y, Akamatsu R. Status of Nutrition Education Using the Three-Color Classification System for Food Groups in Elementary Schools. Jpn J Nutr Diet 75: 91-97. 2017. (in Japanese)
35) Birch LL, McPhee L, Shoba BC, Pirok E, Steinberg L. What kind of exposure reduces children's food neophobia? Looking vs. tasting. Appetite 9: 171-178. 1987.
36) Kherkheulidze M, Kavlashvili N, Kandelaki E, Manjavidze T. Evaluation of nutritional knowledge of second grade school children and assessment of their dietary intake. Georgian Med News (212): 58-64. 2012.
37) Nakanishi A, Takemi Y. Developing a media literacy nutrition education program for school children: a trial for sixth-grade children at an elementary school in Tokyo. Jap J Sch Health 52: 454-464. 2011. (in Japanese)
38) Sato T. Human Nutrition Lecture Series Introduction to Randomized Clinical Trials. Part 1: Non-Randomized Clinical Trials. Jpn J Nutr Diet. 65: 85-89. 2007. (in Japanese)
39) Kline P. The Handbook of Psychological Testing. 1993. Psychology Press London.
40) Urbuna S. Essentials of Psychological Testing. 2004. John Wiley and Sons Inc. New York City.
41) Zarnowiecki D, Sinn N, Petkov J, Dollman J. Parental nutrition knowledge and attitudes as predictors of 5-6-year-old children's healthy food knowledge. Public Health Nutr 15: 1284-1290. 2012.
42) Parmenter K, Waller J, Wardle J. Demographic variation in nutrition knowledge in England. Health Educ Res 15: 163-174. 2000.
43) Hendrie GA, Coveney J, Cox D. Exploring nutrition knowledge and the demographic variation in knowledge levels in an Australian community sample. Public Health Nutr 11: 365-1371. 2008.

Letter to the Editor

Aetiology of Kwashiorkor then and now – still the Deposed Child?

Dear Editor:

Kwashiorkor, described by Cecily Williams in 1933 as the disease of the child “deposed” from breastfeeding, still poses an enigma to scientists today as it did then. Almost nine decades after it was described, there has been a decline in prevalence in some parts of the world, but it remains prevalent in other parts, especially in East Africa where it is reported to be more common than marasmus (1). Changes in the nomenclature and current treatment modalities make it seem as if the two conditions are the same, though distinct pathophysiological differences exist between the two conditions (2). Kwashiorkor is a multisystem disease, characterised by oedema and multiorgan dysfunction resulting from the body’s inability to maintain cell membrane integrity, leading to potassium and water loss from all categories of cells. It is associated with consumption of monotonous diets, consisting mainly of maize, cassava and rice (3). Pathological changes such as deposition of fat in the liver, villous atrophy and anaemia have been observed. However, the primary aetiology is largely unknown at this time, but the search goes on.

Several factors are implicated in the aetiology of kwashiorkor, though many have been disputed. These include prolonged breast feeding, inadequate protein intake, hypoalbuminemia, intoxication from aflatoxins, excessive oxidant stress and measles infection. (3) More recent animal studies have shown that hepatic steatosis or the fatty liver changes in kwashiorkor can be prevented by feeding mice with a maize vegetable diet supplemented with choline, suggesting that choline insufficiency maybe a contributory factor (4). Others have observed DNA hypomethylation and linked this to the slow turnover of 1-carbon cycle metabolites such as methionine (5). An increased proportion of proteobacteria and more frequent cultures of fusobacterium from gut microbiota have been found in Kwashiokor compared to marasmus, supporting hypothesis that gut microbiota produce toxins that cause the cell damage. (6) Studies have shown low levels of antioxidants such as beta carotene in diets of these children (7).

One hypothesis that remains difficult to discard is the role of inadequate protein intake and hypoalbuminemia in the aetiology. Semba in his narrative on “The Rise and Fall of Protein Malnutrition” provides a succinct historical account of global efforts made in the past to support this theory, close the protein gap and the controversies that ensued. It is striking to note that today, stunting which is a milder form of malnutrition that affects millions of children globally, and considered by some to be a pre-morbid state of kwashiorkor has been linked to inadequate protein intake with correctional efforts aimed at improving protein intake [8]. So perhaps it is time to reconsider the role of protein or its components in the aetiology of Kwashiorkor.

Thus, although significant progress has been made since Dr Cecily William’s description of Kwashiokor in 1933, the aetiology remains elusive, probably because there are several causal factors using different pathways or influencing a common pathway. Current advances in medicine and genetic sequencing, more than ever, provide a unique opportunity to investigate proposed hypotheses, resolve controversies and discover new concepts about undernutrition and disease conditions associated with kwashiorkor and marasmus.

Edem M. A Tette[1],Juliana YarteyEnos[2*]

[1]Department of Community Health, University of Ghana Medical School
[2] Noguchi Memorial Institute for medical Research, University of Ghana
* Corresponding Author

REFERENCES

1) Williams C. A nutritional disease of children associated with a maize diet. Arch Dis Childh. 1933;8:423–428.
2) Ndekha M J. Kwashiorkor and severe acute malnutrition in childhood, Lancet 2008; 371(9626):1748 DOI: https://doi.org/10.1016/S0140-6736(08)60752-7
3) Manary MJ, Heikens GT, Golden M. Kwashiorkor: more hypothesis testing is needed to understand the aetiology of oedema. *Malawi Med J*. 2009;21(3):106-7. doi: 10.4314/mmj.v21i3.45630. PMID: 20345018; PMCID: PMC3717490.
4) May T, Klatt KC, Smith J, Castro E, Manary M, Caudill MA, Jahoor F, Fiorotto ML. Choline supplementation prevents a hallmark disturbance of Kwashiorkor in weanling mice fed a maize vegetable diet: Hepatic steatosis of undernutrition. *Nutrients*. 2018, 22:10(5):653. doi: 10.3390/nu10050653.
5) Schulze KV, Swaminathan S, Howell S, Jajoo A, Lie NC, Brown O et al Edematous severe acute malnutrition is characterized by hypomethylation of DNA. Nat Commun. 2019;10(1):5791. doi: 10.1038/s41467-019-13433-6
6) Pham TP, Tidjani Alou M, Bachar D, Levasseur A, Brah S, Alhousseini D, Sokhna C, Diallo A, Wieringa F, Million M, Raoult D. Gut Microbiota Alteration is Characterized by a Proteobacteria and Fusobacteria Bloom in Kwashiorkor and a Bacteroidetes Paucity in Marasmus. *Sci Rep*. 2019 Jun 24;9(1):9084. doi: 10.1038/s41598-019-45611-3.

7) Kismul H, Van den Broeck J, Lunde TM. Diet and kwashiorkor: a prospective study from rural DR Congo. Peer J 2014; 2: e350
8) Semba RD. The Rise and Fall of Protein Malnutrition in Global Health. *Ann NutrMetab*. 2016;69(2):79–88. doi:10.11 59/000449175Coulthard MG. Oedema in kwashiorkor is caused by hypo-albuminaemia. *Paediatr Int Child Health*. 2015, 35(2):83-9. doi: 10.1179/204690551 4Y.0000000154.