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Cocoa Relieves Children's Stress: Randomized Parallel-group Comparison Study

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Abstract

This study aimed to determine the effects of consuming cocoa drinks on stress levels and physiological conditions of children. This single-blind, non-intervention controlled, parallel group comparison study recruited 44 children aged 12 years who were healthy but had stress. Blood tests were conducted to ensure safety and to explore any potential effect, and stress was evaluated using the General Health Questionnaire (GHQ). The participants were randomized into two groups: the cocoa group, who consumed cocoa drink for four weeks, and the control group. The study was conducted between October and November 2022. We analyzed the GHQ and blood test results of 37 children. There was a significant improvement in stress level from GHQ, and in serum Dihomo-γ-linolenic acid (DGLA) of children in the cocoa group compared to those in the control group. Consuming cocoa drinks for four weeks showed lowered stress levels in children.

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Keywords: children's stress; cocoa drink; GHQ; DGLA; randomized controlled trial

Introduction

Children's mental health is important for their overall health and wellbeing. The percentage of children and adolescents of age 6–17 years diagnosed with either anxiety or depression increased from 5.4% in 2003 to 8.4% in 2012 in the US [1]. During the COVID-19 pandemic, children's mental health crisis reached the maximum level [2].

In Japan, approximately 10% of adolescents were reported to have moderate-to-severe depressive symptoms in 2019 [3]. A global meta-analysis of children's mental health reported that 25% of children showed a depressive state [4].

In March 2023, a study on suicide among Japanese children in junior to high school reported that 512 children committed suicide in 2022—the highest record in history in Japan [5].

Medical professionals are advising that children, parents, and school teachers should seek help and treatment; however, in Japan, only 25–29% of 11–12-year-olds with depressive symptoms seek help [6]. The reasons for this are mentioned as "wanted to wait and see" and "did not know where to go." [6]

Because many children suffer from mental vulnerability, supportive measures within and outside of the home are needed to mitigate their stress and help them acquire resilience. The Centers for Disease Control and Prevention (CDC) in the US shared access information to mental health treatment [6]. American Psychological Association is training school teachers in basic social and emotional skills to help students cope with stress and anxiety at school [7]. The research team at the National Center for Child Health and Development in Japan is proposing safety nets in each level at home, schools, and community [8]. State-lead or community-based safeguard and safety nets are important, but small scale measures at home as a gentle preventive step are also expected. Food, nutrition, and lifestyle changes are potential measures to avoid depressive state and relieve stress in children at home, school, and in society. A study from Belgium shows the role of food including cocoa in stress reduction among children [9]. There have been multiple studies that indicate effect of chocolate intake on psychosocial stress in adults [10-12]. There have also been studies of influence of chocolate intake on mood [13-14].

This study aimed to assess the effects of drinking commercially available premixed cocoa at home on the mental state of children who are prone to stress as a simple, affordable, and accessible way to alleviate stress and achieve peace of mind.

Materials and methods

Design

This was a prospective, single-center, two-arm, parallel-group, randomized parallel-group comparison trial. The study protocol was approved by the Institutional Review Board of Japan Conference of Clinical Research (IRB No. 2022-001) on October 3, 2022. The trial

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was registered as a randomized parallel-group comparison trial titled "A study to confirm the effects of test food consumption on mental health and nutritional status during the growth period" with the University hospital Medical Information Network (UMIN) registration number UMIN 000048794. This study was conducted in accordance with the World Medical Association Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology, and Ministry of Health, Labour and Welfare, Japan). In addition, this study conformed to the CONSORT 2010 statement. Written informed consent was obtained from each participant's parent prior to the study.

Participants

We recruited 44 participants in October 2022 through 3H Medi Solution, Inc. We aimed for a sample size of more than 30, at least 15 in each arm. The number was drawn as a feasible number to recruit children who would agree to an invasive blood test, also a minimum number required to achieve statistical effective result in a normally distributed population. The inclusion criteria were: healthy children aged 12 years at the time of registration; body mass index (BMI) between 17–27.0 kg/m^2 in girls and 16.5–27.0 kg/m^2 in boys; body weight between 35–55 kg; and experiencing some stress. The exclusion criteria were: those with body bass index (BMI) lower than 17 in girls, lower than 16.5 in boys; body weight being lower than 35 kg or higher than 55 kg; those who turned positive in Covid-19 or any other infectious diseases at the time of the trial; and children who regularly visited medical institutions for any medical condition.

Upon registration for this trial, the parent/guardian was asked to report on the stress level of each child as response to the written question "This trial is seeking for 12-year-old healthy children but with some stress." We chose 12 years as the target age because children of this age are considered prone to stress in the Japanese society as they are in their final year of elementary school and are preparing for entrance examinations for junior high schools, which can be quite competitive. Additionally, the age indicates the beginning of adolescence. At this age, a child can participate in a clinical trial proactively at their own will and with good understanding. 38 participants attended the trial. After we explained the study to them, all 38 participants signed the consent form to participate in the study at their free will.

Table 1. *Anthropometric attributes of the study participants*

	Total (n=38)	Intervention group (n=18)	Control group (n=20)	P value
Age	12	12	12	1.000
Gender				
Female	20	9	11	
Male	18	9	9	
Body height (avg, SD) Total		150.46±6.57	150.70±6.95	0.914
Female		152.38±4.24	148.43±5.23	0.085
Male		148.54±8.09	153.48±8.06	0.213
Body weight (ave, SD) Total		44.16±6.02	44.40±6.63	0.906
Female		45.47±5.30	44.25±7.96	0.698
Male		42.84±6.70	44.59±5.01	0.541
BMI Total		19.49±2.18	19.53±2.59	0.969
Female		19.59±2.11	20.02±3.11	0.729
Male		19.40±2.37	18.92±1.78	0.635

Measurements

All participants and their parents/guardians went through all the tests including blood test in Akasaka Family Clinic, Tokyo. All participants completed the General Health Questionnaire (GHQ; Nakagawa, Daibo©, Nihon Bunka Kagakusha Company Limited, Tokyo, 2013), the Japanese version of which is applicable from age 12 and higher, and their parents completed the Pediatric Symptom Checklist 17 Japanese version (J-PSC 17). The participants underwent body composition measurements, blood pressure measurements, blood tests, and medical examinations, all of which were used as baseline assessments before the intervention. The items of the blood test are; white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hematocrit (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, sodium, chloride, calcium, phosphorous, magnesium, iron, ferritin, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride, total protein, albumin, aspartate transaminase, alanine transaminase, alkaline phosphatase, blood urea nitrogen, creatinine, uric acid, hemoglobin A1c (HbA1c), 25-hydroxyvitamin D, zinc, copper, dihomo-gamma-linoleic acid (DGLA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA). Two participants were excluded based on the exclusion criteria. Figure 1 shows the study flow diagram. The same set of tests was conducted as the "endpoint assessment" after the 4-week intervention period. Block-design randomization was applied based on sex, body mass index (BMI), total protein, total cholesterol, and LDL cholesterol levels. Thirty-six participants were enrolled in the trial: 18 each in the intervention and control groups, with 9 girls and 9 boys in each group. Allocation of participants, final decision for enrollment, and assignment of participants in each group were conducted by the supervising physician of this trial.

Procedure

Readily available premixed cocoa powder, produced by Morinaga & Co., Ltd., was used as the intervention food because it is one of the most popular cocoa products consumed by children in Japan. A cocoa drink containing 24 g of premixed cocoa powder dissolved in 120 mL of hot or cold water was consumed daily for four weeks. The time of consumption within the day was sufficiently flexible to facilitate drinking. The nutritional composition of the premixed cocoa powder is shown in Table 3. Cow's milk was not to be used for the drink because Japanese children of this age are usually served one bottle (approximately 200 ml) of cow's milk at school during lunch; a higher consumption of it may cause milk anemia, an iron-deficient anemic state due to iron absorption inhibitive features of calcium and casein contained in cow's milk and due to the low iron content in cow's milk [15]. The control group were not supplied with any placebo food. None of the participants were requested to consume chocolate, drinks, or any food that included cacao or cocoa.

Outcome

The GHQ was developed as a self-administered screening tool for psychological distress to be used in general practice or in primary care settings [16] It has been translated into 38 languages and its validity has been indicated [17]. GHQ-12

Table 2. General Health Questionnaire: GHQ-12: positively phrased and negatively phrased questions

Positively phrased items	Negatively phrased items
Able to concentrate	Lost sleep over worry
Felt playing useful part in things	Felt constantly under strain
Felt capable of making decisions	Felt could not overcome difficulties
Able to enjoy day-to-day activities	Been feeling unhappy and depressed
Been able to face problems	Been losing confidence in self
Feeling reasonably happy	Been thinking of self as worthless

Source: An Assessment of the Psychometric Properties of the GHQ-12 in an English Population of Autistic Adults Without Learning Difficulties

is a shorter version comprising 12 simple questions to screen for risk of common mental disorders and minor psychological problems [18,19]. We used GHQ-12 to measure the primary outcome because the test is simple to administer and is validated for ages 12 and above in the Japanese version [20]. The Japanese version of the GHQ-12 was used (Table 2). The test results were calculated using the GHQ standard binary score method (0-0-1-1).

Statistical analysis

For statistical analysis, we used t-tests to compare the averages of continuous variables, and Wilcoxon tests to compare the averages of non-parametric data, such as the GHQ test results. The significance level was set at $P<0.05$. The statisticians evaluating the study results were blinded to the groups. To avoid bias, the first author (HI) did not participate in any statistical analyses.

All statistical analyses were performed using SPSS Ver. 26 (IBM), STATA Ver. 17, SAS.

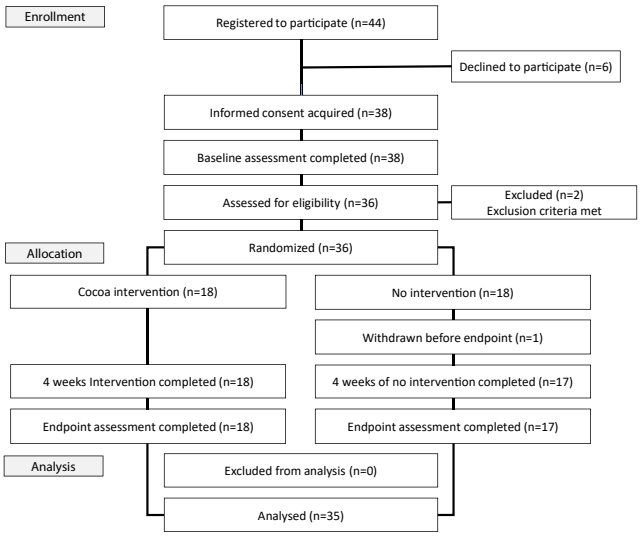

Figure 1. *Flow diagram of participants' progress through enrollment to analysis*

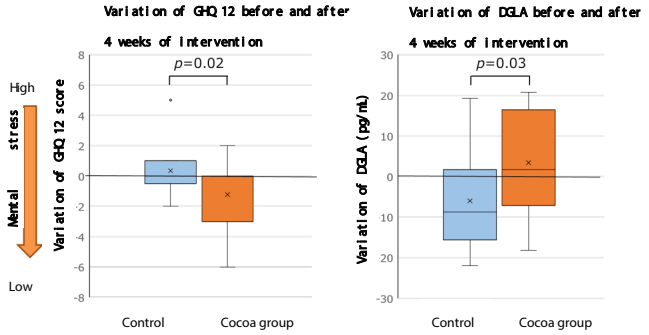

Figure 2. *Variation of GHQ 12, and of DGLA before and after 4 weeks of intervention*

Table 3. *Pediatric Symptom Checklist 17 Japanese version J-PSC 17*

No.	Item	Answers		
		Never	Sometimes	Often
1	Feel sad.			
2	Feel hopeless.			
3	Feel down on him/herself.			
4	Worry a lot.			
5	Seem to be having less fun.			
6	Fidget, is unable to sit still.			
7	Daydream too much.			
8	Distract easily.			
9	Have trouble concentrating.			
10	Act as if driven by a motor.			
11	Fight with other children.			
12	Not listen to rules.			
13	Not understand other people's feelings.			
14	Tease others.			
15	Blame others for his/her troubles.			
16	Refuse to share.			
17	Take things that do not belong to him/her.			
*The above is the original English version. The Japanese translated version was used in our study.				

Results

After four weeks of intervention, one participant dropped out of the control group, resulting in 18 participants in the intervention and 17 in the control group (Figure 1). Adherence to cocoa intake was 98.8%, calculated from the daily record sheet maintained by the participants and their parents/guardians during the trial. The primary endpoint GHQ-12 showed a significant decrease in the cocoa group (cocoa group, standard deviation (SD) -1.2±2.2, control group SD 0.4±1.5, P=0.046) (Table 5, Figure 2). There was no significant difference between the two groups in the J-PSC 17 administered to the parents/guardians. Height in girls in cocoa group (9 girls) increased

Table 4. Ingredients of Morinaga adjusted cocoa powder

Sugar Cocoa powder (cocoa butter 22 ~ 24%) Glucose Skimmed milk powder Lactose Creaming powder Maltose Whole milk powder Cocoa mass Salt Flavoring pH adjuster emulsifier
Polyphenol 200 mg
Per 24 g/cup Energy 77 kcal Protein 1.9 g Lipid 1.6 g
Carbohydrate 15.1 g Sugar 13.8 g Dietary fiber 1.3 g
Salt equivalent 0.09 g

significantly compared to 8 girls in the control group (SD 0.73±0.23, P=0.031). Moreover, in girls, body weight increased significantly in cocoa group compared to the control group (SD 0.9±0.61, P=0.013). The test results before and after the intervention for both boys and girls are presented in Table 5, subgroup analysis for boys in Table 7, subgroup analysis for girls in Table 8. The results of the analysis showed that DGLA increased significantly in the cocoa group compared to the control group (cocoa group: SD 3.39±12.69, control group: SD -6.00±11.24, P=0.027) (Table 5, Figure 2). Uric acid increased in the girls in cocoa group (SD 0.14±0.29, P=0.014) and ferritin decreased among girls after the intervention (SD -10.4±6.4, P=0.014) (Table 8). Analysis of variance (ANOVA) inter-group analysis showed that ferritin decreased more in cocoa group (confidence interval [CI]=0.29–9.09, P=0.037; Table 6). In girls, the DHA/AA and (EPA+DHA)/AA ratios showed significant differences before intervention between the two groups (DHA/AA ratio: cocoa group SD 0.526±0.128, control group SD 0.36±0.103, P=0.005; (EPA+DHA)/AA ratio: cocoa group SD 0.068±0.223, control group SD 0.449±0.146, P=0.016) (Table 8). There was no significant difference between the two groups after the intervention.

Table 5. *Test results before and after intervention*

Test items		Before intervention		t-test/	After intervention		t-test/	After - Before		t-test/
		Cocoa n=18	Control n=20	Wilcoxon	Cocoa n=18	Control n=17	Wilcoxon	Cocoa n=18	Control n=17	Wilcoxon
BH Ave (SD)		18 150.46±6.57	20 150.70±6.95	p=0.914	18 151.51±6.45	17 151.46±7.81	p=0.985	18 1.04±0.64	17 0.77±0.62	p=0.210
BW Ave (SD)		18 44.16±6.02	20 44.40±6.63	p=0.906	18 45.28±5.47	17 43.25±5.40	p=0.276	18 1.13±0.87	17 0.83±1.10	p=0.380
BMI Ave (SD)		18 19.49±2.18	20 19.53±2.59	p=0.969	18 19.74±2.08	17 18.83±1.53	p=0.153	18 0.24±0.35	17 0.18±0.41	p=0.604
GHQ12 Ave (SD)	n	18 2.5±3.0	20 1.2±1.5	p=0.075	18 1.3±1.6	17 1.6±1.9	p=0.571	18 -1.2±2.2	17 0.4±1.5	p=0.046
J-PSC17 Ave (SD)	n	18 11.1±7.0	20 8.3±6.8	p=0.168	18 9.6±6.6	17 7.2±6.4	p=0.197	18 -1.5±5.5	17 -0.6±3.6	p=0.790
WBC Ave (SD)	n	18 6033.3±1284.8	20 6260.0±1865.6	p=0.669	18 5483.3±913.5	17 5400.0±1278.2	p=0.825	18 -550.0±977.5	17 -776.5±1503.1	p=0.599
RBC Ave (SD)	n	18 492.9±33.6	20 472.9±37.1	p=0.091	18 489.2±31.8	17 473.9±43.3	p=0.242	18 -3.8±18.7	17 4.4±26.2	p=0.292
Hb Ave (SD)	n	18 14.12±1.02	20 13.69±0.84	p=0.156	18 13.98±0.89	17 13.75±1.01	p=0.467	18 -0.14±0.48	17 0.05±0.64	p=0.336
Ht Ave (SD)	n	18 42.96±2.73	20 41.41±2.28	p=0.064	18 42.27±2.57	17 41.13±3.15	p=0.247	18 -0.69±1.58	17 -0.22±1.94	p=0.441
MCV Ave (SD)	n	18 87.2±2.9	20 87.8±3.3	p=0.596	18 86.5±2.9	17 87.0±3.3	p=0.656	18 -0.7±1.0	17 -1.3±2.2	p=0.370
MCH Ave (SD)	n	18 28.67±1.12	20 29.01±1.24	p=0.394	18 28.61±1.12	17 29.08±1.13	p=0.219	18 -0.07±0.49	17 -0.14±0.72	p=0.720
MCHC Ave (SD)	n	18 32.88±0.99	20 33.04±0.68	p=0.569	18 33.08±0.66	17 33.44±0.50	p=0.081	18 0.20±0.74	17 0.32±0.71	p=0.634
Plt Ave (SD)	n	18 31.41±6.20	20 30.32±5.91	p=0.582	18 30.49±6.43	17 30.24±5.68	p=0.903	18 -0.92±2.57	17 -0.20±3.94	p=0.525
Na Ave (SD)	n	18 136.6±2.2	20 137.2±1.9	p=0.371	18 133.9±2.2	17 135.0±2.8	p=0.197	18 -2.7±2.5	17 -2.5±2.5	p=0.820
Cl Ave (SD)	n	18 103.7±1.7	20 103.8±1.7	p=0.810	18 103.8±1.4	17 104.6±1.6	p=0.119	18 0.2±1.9	17 0.7±2.2	p=0.441
Ca Ave (SD)	n	18 9.32±0.30	20 9.27±0.42	p=0.699	18 9.17±0.25	17 9.08±0.25	p=0.298	18 -0.14±0.26	17 -0.18±0.38	p=0.774

Test items		Before intervention		t-test/	After intervention		t-test/	After - Before		t-test/
		Cocoa n=18	Control n=20	Wilcoxon	Cocoa n=18	Control n=17	Wilcoxon	Cocoa n=18	Control n=17	Wilcoxon
P	n	18	20	p=0.969	18	17	p=0.812	18	17	p=0.487
Ave (SD)		4.47±0.53	4.46±0.50		4.73±0.64	4.68±0.46		0.26±0.28	0.18±0.38	
Mg	n	18	20	p=0.404	18	17	p=0.420	18	17	p=0.291
Ave (SD)		2.29±0.11	2.27±0.10		2.23±0.10	2.26±0.08		-0.06±0.12	-0.02±0.08	
Fe	n	18	20	p=0.195	18	17	p=0.620	18	17	p=0.286
Ave (SD)		111.4±29.9	98.0±32.6		103.1±46.0	109.8±31.4		-8.3±46.7	8.0±41.9	
Ferritin	n	18	20	p=0.964	18	17	p=0.359	18	17	p=0.054
Ave (SD)		28.8±16.6	28.5±17.6		20.8±12.8	26.0±19.5		-8.0±7.5	-3.4±6.0	
TCho	n	18	20	p=0.164	18	17	p=0.116	18	17	p=0.950
Ave (SD)		168.4±19.0	157.6±26.7		170.6±21.2	158.5±23.3		2.2±13.5	2.5±15.2	
HDL	n	18	20	p=0.477	18	17	p=0.460	18	17	p=0.605
Ave (SD)		60.8±11.1	58.5±9.3		61.9±9.7	59.2±11.3		1.1±5.5	0.0±6.5	
LDL	n	18	20	p=0.346	18	17	p=0.245	18	17	p=0.758
Ave (SD)		97.8±19.0	90.8±25.2		95.4±21.2	87.2±20.1		-2.3±9.4	-1.2±11.4	
TG	n	18	19	p=0.585	18	17	p=0.230	18	16	p=0.725
Ave (SD)		74.0±41.5	67.3±31.8		81.9±55.1	63.6±29.1		7.9±54.7	2.3±33.9	
TP	n	18	20	p=0.048	18	17	p=0.127	18	17	p=0.227
Ave (SD)		7.47±0.39	7.23±0.34		7.44±0.41	7.26±0.28		-0.03±0.26	0.08±0.24	
Alb	n	18	20	p=0.077	18	17	p=0.418	18	17	p=0.161
Ave (SD)		4.73±0.22	4.59±0.24		4.66±0.23	4.60±0.22		-0.07±0.22	0.03±0.16	
AST	n	18	20	p=0.845	18	17	p=0.937	18	17	p=0.779
Ave (SD)		19.9±5.8	20.2±3.8		22.0±6.7	22.2±6.3		2.1±4.9	1.7±3.4	
ALT	n	18	20	p=0.705	18	17	p=0.882	18	17	p=0.704
Ave (SD)		13.3±5.6	14.0±5.1		15.1±8.0	14.8±5.2		1.8±7.3	1.1±2.7	
ALP	n	18	20	p=0.846	18	17	p=0.269	18	17	p=0.120
Ave (SD)		289.4±127.6	282.0±107.9		253.8±83.1	290.9±111.4		-35.7±88.7	1.3±36.6	
BUN	n	18	20	p=0.647	18	17	p=0.535	18	17	p=0.747
Ave (SD)		11.86±2.41	12.17±1.69		11.98±2.39	12.45±2.07		0.12±2.33	0.38±2.28	
Crea	n	18	20	p=0.513	18	17	p=0.999	18	17	p=0.087
Ave (SD)		0.499±0.081	0.515±0.064		0.511±0.071	0.511±0.047		0.011±0.026	-0.011±0.045	
UA	n	18	20	p=0.136	18	17	p=0.699	18	17	p=0.133
Ave (SD)		4.77±0.94	5.30±1.15		4.81±0.76	4.93±1.02		0.04±0.81	-0.31±0.48	
HbA1c	n	18	20	p=0.425	18	17	p=0.197	18	17	p=0.682
Ave (SD)		5.18±0.23	5.11±0.28		5.22±0.21	5.12±0.24		0.04±0.21	0.06±0.15	
25-OHVD	n	18	20	p=0.393	18	17	p=0.258	18	17	p=0.738
Ave (SD)		19.41±6.15	20.94±4.77		15.68±5.78	18.02±6.27		-3.73±3.12	-3.36±3.34	
Zn	n	18	20	p=0.603	18	17	p=0.491	18	17	p=0.732
Ave (SD)		103.8±18.4	101.1±14.2		104.5±14.5	101.1±14.8		0.7±14.1	-0.8±11.1	
Cu	n	18	20	p=0.530	18	17	p=0.721	18	17	p=0.712
Ave (SD)		91.7±13.8	95.0±17.6		93.7±14.9	95.9±22.0		1.9±10.3	3.6±15.4	
DGLA	n	18	20	p=0.230	18	17	p=0.025	18	17	p=0.027
Ave (SD)		31.88±7.13	34.62±6.70		35.27±7.71	29.36±7.14		3.39±12.69	-6.00±11.24	
AA	n	18	20	p=0.413	18	17	p=0.252	18	17	p=0.204
Ave (SD)		156.32±29.39	165.07±35.07		162.72±25.35	150.88±34.23		6.40±40.84	-13.35±49.18	
EPA	n	18	20	p=0.083	18	17	p=0.805	18	17	p=0.231
Ave (SD)		22.77±21.39	13.69±7.53		22.50±15.18	21.28±13.85		-0.27±28.36	8.97±13.39	
DHA	n	18	20	p=0.053	18	17	p=0.303	18	17	p=0.636
Ave (SD)		74.6±14.9	62.9±20.3		72.1±27.0	63.9±18.0		-2.6±33.0	2.1±24.2	
EPA/AA	n	18	20	p=0.042	18	17	p=0.788	18	17	p=0.293
Ave (SD)		0.128±0.087	0.082±0.044		0.150±0.099	0.141±0.094		0.022±0.153	0.067±0.088	
DHA/AA	n	18	20	p=0.519	18	17	p=0.959	18	17	p=0.478
Ave (SD)		0.492±0.130	0.752±1.686		0.439±0.138	0.441±0.123		-0.053±0.208	-0.371±1.868	
(EPA+DHA)/AA	n	18	20	p=0.009	18	17	p=0.956	18	17	p=0.083
Ave (SD)		0.620±0.208	0.464±0.139		0.567±0.220	0.571±0.193		-0.053±0.344	0.120±0.210	

Wilcoxon was used for GHQ 12 and for J-PSC 17.
T-test was used for all other test analysis.

Ave, Average; SD, Standard Deviation; BH, Body Height; BW, Body Weight; BMI, Body Mass Index; GHQ12, General Health Questionnaire 12; J-PSC17, Japanese version Pediatric Stress Checklist 17; WBC, White Blood Cell; RBC, Red Blood Cell; Hb Hemoglobin; Ht, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hematocrit; MCHC, Mean Corpuscular Hemoglobin Concentration; Plt, Platelet; Na, Sodium; Cl, Chloride; Ca, Calcium; P, Phosphorus; Mg, Magnesium; Fe, Serum Iron; Ferritin, Ferritin; TCho, Total Cholesterol; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; TG, Triglyceride; TP, Total Protein; Alb, Albumin; AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase; BUN, Blood Urea Nitrogen; Crea, Creatinine; UA, Uric Acid; HbA1c, Hemoglobin A1c; 25-OHVD, 25-hydroxy vitamin D; Zn, Zinc; Cu, Copper; DGLA, Dihomo-gamma Linoleic Acid; AA, Arachidonic Acid; EPA, Eicosapentaenoic Acid; DHA, Docosahexiaenoic Acid.

Table 6. *ANOVA between the cocoa group and control group Attributes, test results*

	Least squares mean		Difference of least squares mean	95% CI	P value
	Cocoa	Control			
BH	1.05	0.77	-0.28	-0.72 ~ 0.17	p=0.212
BW	1.15	0.81	-0.34	-1.03 ~ 0.36	p=0.335
BMI	0.26	0.15	-0.11	-0.38 ~ 0.16	p=0.408
GHQ12	-0.90	0.01	0.91	-0.07 ~ 1.89	p=0.067
J-PSC17	-1.05	-1.12	-0.07	-3.10 ~ 2.96	p=0.963
WBC	-589.34	-734.81	-145.47	-730.84 ~ 439.90	p=0.616
RBC	-2.50	3.06	5.57	-10.95 ~ 22.09	p=0.497
Hb	-0.10	0.01	0.11	-0.27 ~ 0.50	p=0.563
Ht	-0.60	-0.31	0.29	-0.99 ~ 1.57	p=0.649
MCV	-0.79	-1.22	-0.43	-1.62 ~ 0.77	p=0.474
MCH	-0.10	-0.11	-0.01	-0.44 ~ 0.42	p=0.971
MCHC	0.13	0.39	0.27	-0.08 ~ 0.62	p=0.128
Plt	-0.84	-0.28	0.56	-1.65 ~ 2.76	p=0.609
Na	-2.90	-2.23	0.67	-0.99 ~ 2.33	p=0.417
Cl	0.06	0.82	0.76	-0.27 ~ 1.80	p=0.141
Ca	-0.13	-0.20	-0.07	-0.22 ~ 0.08	p=0.353
P	0.26	0.18	-0.08	-0.30 ~ 0.15	p=0.508
Mg	-0.06	-0.03	0.03	-0.03 ~ 0.09	p=0.294
Fe	-5.00	4.53	9.53	-17.75 ~ 36.81	p=0.482
Ferritin	-8.06	-3.36	4.69	0.29 ~ 9.09	p=0.037
TCho	3.39	1.29	-2.10	-12.08 ~ 7.88	p=0.671
HDL	1.18	-0.13	-1.32	-5.33 ~ 2.70	p=0.509
LDL	-1.73	-1.88	-0.15	-7.39 ~ 7.09	p=0.967
TG	10.86	-0.97	-11.82	-41.98 ~ 18.33	p=0.430
TP	0.01	0.04	0.03	-0.15 ~ 0.21	p=0.738
Alb	-0.03	0.00	0.03	-0.10 ~ 0.16	p=0.628
AST	2.11	1.71	-0.40	-3.37 ~ 2.57	p=0.787
ALT	1.74	1.10	-0.63	-4.41 ~ 3.14	p=0.736
ALP	-35.70	1.33	37.03	-2.49 ~ 76.55	p=0.065
BUN	0.06	0.44	0.37	-1.03 ~ 1.78	p=0.594
Crea	0.01	-0.01	-0.02	-0.04 ~ 0.01	p=0.143
UA	-0.03	-0.24	-0.21	-0.65 ~ 0.23	p=0.344
HbA1c	0.06	0.04	-0.02	-0.14 ~ 0.10	p=0.724
25-OHVD	-3.81	-3.27	0.54	-1.72 ~ 2.81	p=0.628
Zn	1.06	-1.24	-2.29	-9.78 ~ 5.19	p=0.537
Cu	1.91	3.63	1.72	-7.30 ~ 10.74	p=0.700
DGLA	0.99	-3.45	-4.44	-9.38 ~ 0.51	p=0.077
AA	2.43	-9.14	-11.57	-32.70 ~ 9.56	p=0.273
EPA	5.21	3.17	-2.04	-12.75 ~ 8.67	p=0.701
DHA	3.86	-4.69	-8.55	-25.86 ~ 8.76	p=0.322

	Least squares mean		Difference of least squares mean	95% CI	P value
	Cocoa	Control			
EPA/AA	0.05	0.03	-0.02	-0.09 ~ 0.05	p=0.524
DHA/AA	-0.21	-0.20	0.01	-0.08 ~ 0.10	p=0.845
(EPA+DHA)/AA	0.04	0.02	-0.03	-0.19 ~ 0.13	p=0.739

Ave, Average; SD, Standard Deviation; BH, Body Height; BW, Body Weight; BMI, Body Mass Index; GHQ12, General Health Questionnaire 12; J-PSC17, Japanese version Pediatric Stress Checklist 17; WBC, White Blood Cell; RBC, Red Blood Cell; Hb Hemoglobin; Ht, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hematocrit; MCHC, Mean Corpuscular Hemoglobin Concentration; Plt, Platelet; Na, Sodium; Cl, Chloride; Ca, Calcium; P, Phosphorus; Mg, Magnesium; Fe, Serum Iron; Ferritin, Ferritin; TCho, Total Cholesterol; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; TG, Triglyceride; TP, Total Protein; Alb, Albumin; AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase; BUN, Blood Urea Nitrogen; Crea, Creatinine; UA, Uric Acid; HbA1c, Hemoglobin A1c; 25-OHVD, 25-hydroxy vitamin D; Zn, Zinc; Cu, Copper; DGLA, Dihomo-gamma Linoleic Acid; AA, Arachidonic Acid; EPA, Eicosapentaenoic Acid; DHA, Docosahexiaenoic Acid

Table 7. *Subgroup Analysis Test Results in Boys*

Test items		Before intervention		T test/ Wilcoxon	After intervention		T test/ Wilcoxon	After-Before		T test/ Wilcoxon
		Cocoa n=9	Control n=9		Cocoa n=9	Control n=9		Cocoa n=9	Control n=9	
BH	n	9	9	p=0.213	9	9	p=0.236	9	9	p=0.514
	Ave (SD)	148.54±8.09	153.48±8.06		149.90±8.09	154.62±8.18		1.36±0.78	1.14±0.54	
BW	n	9	9	p=0.541	9	9	p=0.525	9	9	p=0.952
	Ave (SD)	42.84±6.70	44.59±5.01		44.20±6.00	45.98±5.60		1.36±1.06	1.39±1.26	
BMI	n	9	9	p=0.635	9	9	p=0.627	9	9	p=0.962
	Ave (SD)	19.40±2.37	18.92±1.78		19.69±2.23	19.22±1.73		0.29±0.45	0.30±0.53	
GHQ12	n	9	9	p=0.233	9	9	p=0.814	9	9	p=0.339
	Ave (SD)	2.2±2.4	0.9±1.1		1.1±1.3	1.1±1.2		-1.1±2.5	0.2±0.8	
J-PSC17	n	9	9	p=0.308	9	9	p=0.595	9	9	p=0.562
	Ave (SD)	11.7±5.3	8.9±7.4		7.9±5.3	6.7±4.8		-3.8±6.6	-2.2±3.6	
WBC	n	9	9	p=0.767	9	9	p=0.441	9	9	p=0.709
	Ave (SD)	5633.3±997.5	5477.8±1185.1		5133.3±678.2	4811.1±1019.1		-500.0±1173.7	-666.7±595.8	
RBC	n	9	9	p=0.167	9	9	p=0.659	9	9	p=0.053
	Ave (SD)	506.4±36.3	484.0±29.0		495.9±26.1	488.9±38.7		-10.6±16.1	4.9±15.2	
Hb	n	9	9	p=0.251	9	9	p=0.855	9	9	p=0.074
	Ave (SD)	14.58±1.04	14.09±0.66		14.23±0.79	14.16±0.97		-0.34±0.39	0.07±0.51	
Ht	n	9	9	p=0.247	9	9	p=0.905	9	9	p=0.103
	Ave (SD)	43.77±3.22	42.28±1.85		42.61±2.46	42.46±2.94		-1.16±1.61	0.18±1.67	
MCV	n	9	9	p=0.471	9	9	p=0.516	9	9	p=0.957
	Ave (SD)	86.5±2.4	87.5±3.5		86.0±3.0	87.0±3.5		-0.5±0.9	-0.5±0.8	
MCH	n	9	9	p=0.564	9	9	p=0.608	9	9	p=0.851
	Ave (SD)	28.82±1.31	29.17±1.16		28.72±1.21	29.02±1.23		-0.10±0.63	-0.14±0.30	
MCHC	n	9	9	p=0.974	9	9	p=0.806	9	9	p=0.866
	Ave (SD)	33.33±0.92	33.32±0.43		33.42±0.65	33.36±0.47		0.09±0.93	0.03±0.31	
Plt	n	9	9	p=0.411	9	9	p=0.856	9	9	p=0.151
	Ave (SD)	30.98±7.93	28.49±3.94		29.10±7.70	28.56±4.44		-1.88±2.48	0.07±2.97	
Na	n	9	9	p=0.078	9	9	p=0.111	9	9	p=0.646
	Ave (SD)	136.6±1.5	138.0±1.7		134.4±1.9	136.2±2.5		-2.1±1.7	-1.8±1.3	
Cl	N	9	9	p=0.475	9	9	p=0.175	9	9	p=0.732
	Ave (SD)	103.6±2.1	104.2±1.7		103.9±1.5	104.9±1.5		0.3±2.2	0.7±1.9	
Ca	n	9	9	p=0.606	9	9	p=0.512	9	9	p=0.270
	Ave (SD)	9.24±0.22	9.30±0.22		9.11±0.23	9.04±0.19		-0.13±0.22	-0.26±0.23	
P	n	9	9	p=0.891	9	9	p=0.849	9	9	p=0.899
	Ave (SD)	4.61±0.63	4.58±0.35		4.87±0.80	4.81±0.32		0.26±0.35	0.23±0.38	
Mg	n	9	9	p=0.641	9	9	p=0.379	9	9	p=0.286
	Ave (SD)	2.34±0.10	2.32±0.10		2.24±0.07	2.28±0.08		-0.10±0.13	-0.04±0.07	

Test items		Before intervention		T test/ Wilcoxon	After intervention		T test/ Wilcoxon	After-Before		T test/ Wilcoxon
		Cocoa n=9	Control n=9		Cocoa n=9	Control n=9		Cocoa n=9	Control n=9	
Fe	n	9	9	p=0.270	9	9	p=0.676	9	9	p=0.271
	Ave (SD)	115.4±31.7	97.1±36.2		102.6±36.2	108.7±23.5		-12.9±53.8	11.6±35.3	
Ferritin	n	9	9	p=0.405	9	9	p=0.378	9	9	p=0.869
	Ave (SD)	28.0±13.2	23.9±5.3		22.3±10.2	18.8±5.6		-5.6±8.1	-5.1±5.0	
TCho	n	9	9	p=0.464	9	9	p=0.576	9	9	p=0.753
	Ave (SD)	168.7±18.6	160.3±27.6		164.8±17.0	158.7±27.3		-3.9±12.9	-1.7±16.4	
HDL	n	9	9	p=0.918	9	9	p=0.937	9	9	p=0.782
	Ave (SD)	62.1±12.5	62.7±9.8		61.8±10.8	61.3±12.6		-0.3±7.0	-1.3±8.0	
LDL	N	9	9	p=0.658	9	9	p=0.672	9	9	p=0.894
	Ave (SD)	96.1±21.1	91.0±26.7		90.7±20.3	86.1±24.3		-5.4±9.3	-4.9±8.0	
TG	n	9	9	p=0.470	9	9	p=0.613	9	9	p=0.890
	Ave (SD)	61.4±19.2	53.0±28.4		70.7±60.0	58.7±35.8		9.2±63.8	5.7±40.9	
TP	n	9	9	p=0.083	9	9	p=0.160	9	9	p=0.416
	Ave (SD)	7.38±0.39	7.12±0.14		7.30±0.25	7.14±0.19		-0.08±0.24	0.02±0.27	
Alb	n	9	9	p=0.447	9	9	p=1.000	9	9	p=0.447
	Ave (SD)	4.69±0.23	4.61±0.19		4.61±0.14	4.61±0.22		-0.08±0.25	0.00±0.17	
AST	N	9	9	p=0.865	9	9	p=0.320	9	9	p=0.312
	Ave (SD)	22.6±7.0	22.1±3.3		25.7±7.4	22.8±4.0		3.1±6.8	0.7±1.7	
ALT	n	9	9	p=0.781	9	9	p=0.408	9	9	p=0.516
	Ave (SD)	16.7±6.0	15.9±5.6		19.2±9.7	16.1±5.2		2.6±10.4	0.2±1.6	
ALP	n	9	9	p=0.367	9	9	p=0.544	9	9	p=0.136
	Ave (SD)	385.8±108.5	341.2±94.6		319.8±54.6	342.3±94.6		-66.0±120.6	1.1±43.5	
BUN	n	9	9	p=0.805	9	9	p=0.493	9	9	p=0.336
	Ave (SD)	12.01±2.18	12.28±2.33		13.10±2.21	12.37±2.22		1.09±1.80	0.09±2.43	
Crea	n	9	9	p=0.655	9	9	p=0.872	9	9	p=0.169
	Ave (SD)	0.504±0.109	0.524±0.074		0.521±0.090	0.516±0.047		0.017±0.033	-0.009±0.042	
UA	n	9	9	p=0.498	9	9	p=0.786	9	9	p=0.590
	Ave (SD)	5.18±1.03	5.54±1.20		5.11±0.78	5.24±1.22		-0.07±1.14	-0.30±0.57	
HbA1c	n	9	9	p=0.236	9	9	p=0.576	9	9	p=0.413
	Ave (SD)	5.21±0.25	5.07±0.25		5.23±0.23	5.17±0.26		0.02±0.25	0.10±0.11	
25-OHVD	n	9	9	p=0.598	9	9	p=0.721	9	9	p=0.896
	Ave (SD)	20.89±5.50	22.22±5.00		17.19±6.47	18.36±7.11		-3.70±2.40	-3.87±2.89	
Zn	n	9	9	p=0.398	9	9	p=0.828	9	9	p=0.434
	Ave (SD)	110.2±22.2	103.2±9.7		106.6±15.7	104.9±16.2		-3.7±17.7	1.7±9.1	
Cu	n	9	9	p=0.986	9	9	p=0.980	9	9	p=0.984
	Ave (SD)	89.1±12.4	89.0±14.6		92.4±14.0	92.2±22.5		3.3±9.0	3.2±14.0	
DGLA	n	9	9	p=0.466	9	9	p=0.622	9	9	p=0.483
	Ave (SD)	32.91±4.59	34.56±4.74		31.82±4.51	30.29±7.97		-1.09±8.83	-4.27±9.91	
AA		9	9	p=0.759	9	9	p=0.185	9	9	p=0.351
	Ave (SD)	159.48±36.25	164.56±32.82		157.58±26.09	143.23±16.88		-1.90±48.01	-21.32±37.04	
EPA	n	9	9	p=0.204	9	9	p=0.426	9	9	p=0.784
	Ave (SD)	15.94±7.37	12.04±4.88		24.57±17.97	18.49±13.20		8.62±20.38	6.44±11.56	
DHA	n	9	9	p=0.585	9	9	p=0.284	9	9	p=0.555
	Ave (SD)	70.1±11.8	66.2±17.6		71.9±27.9	60.0±16.1		1.8±33.1	-6.1±21.6	
EPA/AA	n	9	9	p=0.162	9	9	p=0.429	9	9	p=0.925
	Ave (SD)	0.112±0.073	0.073±0.030		0.167±0.122	0.122±0.109		0.054±0.147	0.049±0.094	
DHA/AA	n	9	9	p=0.370	9	9	p=0.600	9	9	p=0.360
	Ave (SD)	0.458±0.131	1.229±2.504		0.467±0.150	0.433±0.112		0.009±0.216	-0.796±2.554	
(EPA+DHA) /AA	N	9	9	p=0.266	9	9	p=0.534	9	9	p=0.853
	Ave (SD)	0.572±0.194	0.481±0.137		0.611±0.257	0.544±0.181		0.039±0.347	0.063±0.176	

Wilcoxon was used for GHQ 12 and for J-PSC 17.
T-test was used for all other test analysis.

Ave, Average; SD, Standard Deviation; BH, Body Height; BW, Body Weight; BMI, Body Mass Index; GHQ12, General Health Questionnaire 12; J-PSC17, Japanese version Pediatric Stress Checklist 17; WBC, White Blood Cell; RBC, Red Blood Cell; Hb Hemoglobin; Ht, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hematocrit; MCHC, Mean Corpuscular Hemoglobin Concentration; Plt, Platelet; Na, Sodium; Cl, Chloride; Ca, Calcium; P, Phosphorus; Mg, Magnesium; Fe, Serum Iron; Ferritin, Ferritin; TCho, Total Cholesterol; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; TG, Triglyceride; TP, Total Protein; Alb, Albumin; AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase; BUN, Blood Urea Nitrogen; Crea, Creatinine; UA, Uric Acid; HbA1c, Hemoglobin A1c; 25-OHVD, 25-hydroxy vitamin D; Zn, Zinc; Cu, Copper; DGLA, Dihomo-gamma Linoleic Acid; AA, Arachidonic Acid; EPA, Eicosapentaenoic Acid; DHA, Docosahexiaenoic Acid.

Table 8. *Subgroup Analysis Test Results in Girls*

Test items		Before intervention		T test/ Wilcoxon	After intervention		T test/ Wilcoxon	After-Before		T test/ Wilcoxon
		Cocoa	Control		Cocoa	Control		Cocoa	Control	
BH	n	n=9	n=9	p=0.085	n=9	n=9	p=0.052	n=9	n=9	p=0.031
	Ave (SD)	152.38±4.24	148.43±5.23		153.11±4.14	147.90±5.97		0.73±0.23	0.35±0.42	
BW	n	9	11	p=0.698	9	8	p=0.009	9	8	p=0.013
	Ave (SD)	45.47±5.30	44.25±7.96		46.37±4.99	40.18±3.22		0.90±0.61	0.20±0.35	
BMI	n	9	11	p=0.729	9	8	p=0.115	9	8	p=0.137
	Ave (SD)	19.59±2.11	20.02±3.11		19.79±2.06	18.39±1.23		0.20±0.24	0.04±0.18	
GHQ12	n	9	11	p=0.182	9	8	p=0.457	9	8	p=0.093
	Ave (SD)	2.8±3.6	1.4±1.8		1.4±1.9	2.3±2.4		-1.3±2.1	0.5±2.1	
J-PSC17	n	9	11	p=0.424	9	8	p=0.210	9	8	p=0.845
	Ave (SD)	10.4±8.7	7.7±6.6		11.2±7.6	7.9±8.2		0.8±3.2	1.1±2.8	
WBC	N	9	11	p=0.583	9	8	p=0.685	9	8	p=0.704
	Ave (SD)	6433.3±1467.1	6900.0±2118.5		5833.3±1018.6	6062.5±1264.8		-600.0±804.7	-900.0±2173.9	
RBC	N	9	11	p=0.341	9	8	p=0.218	9	8	p=0.950
	Ave (SD)	479.4±25.9	463.8±41.6		482.4±36.9	457.1±44.3		3.0±19.5	3.9±36.0	
Hb	N	9	11	p=0.417	9	8	p=0.339	9	8	p=0.897
	Ave (SD)	13.67±0.81	13.35±0.86		13.73±0.95	13.29±0.90		0.07±0.49	0.03±0.79	
Ht	N	9	11	p=0.166	9	8	p=0.112	9	8	p=0.626
	Ave (SD)	42.16±2.00	40.70±2.42		41.93±2.78	39.64±2.83		-0.22±1.50	-0.68±2.23	
MCV	N	9	11	p=0.990	9	8	p=0.962	9	8	p=0.311
	Ave (SD)	88.0±3.3	88.0±3.2		87.0±2.8	86.9±3.4		-1.0±1.1	-2.1±3.0	
MCH	N	9	11	p=0.520	9	8	p=0.230	9	8	p=0.778
	Ave (SD)	28.52±0.95	28.87±1.34		28.49±1.08	29.15±1.10		-0.03±0.33	-0.14±1.03	
MCHC	N	9	11	p=0.319	9	8	p=0.007	9	8	p=0.370
	Ave (SD)	32.42±0.88	32.80±0.77		32.73±0.49	33.53±0.54		0.31±0.53	0.64±0.90	
Plt	N	9	11	p=0.992	9	8	p=0.930	9	8	p=0.775
	Ave (SD)	31.84±4.30	31.82±6.96		31.89±4.92	32.14±6.60		0.04±2.40	-0.50±5.01	
Na	N	9	11	p=0.922	9	8	p=0.812	9	8	p=0.986
	Ave (SD)	136.6±2.8	136.5±1.8		133.3±2.3	133.6±2.6		-3.2±3.2	-3.3±3.3	
Cl	N	9	11	p=0.637	9	8	p=0.449	9	8	p=0.490
	Ave (SD)	103.8±1.2	103.5±1.7		103.8±1.5	104.4±1.7		0.0±1.7	0.8±2.6	
Ca	N	9	11	p=0.506	9	8	p=0.456	9	8	p=0.741
	Ave (SD)	9.39±0.36	9.25±0.54		9.23±0.27	9.13±0.32		-0.16±0.31	-0.09±0.51	
P	N	9	11	p=0.862	9	8	p=0.836	9	8	p=0.364
	Ave (SD)	4.32±0.41	4.36±0.60		4.59±0.44	4.54±0.57		0.27±0.22	0.13±0.39	
Mg	N	9	11	p=0.541	9	8	p=0.775	9	8	p=0.637
	Ave (SD)	2.24±0.10	2.22±0.09		2.22±0.13	2.24±0.07		-0.02±0.11	0.00±0.08	
Fe	n	9	11	p=0.531	9	8	p=0.761	9	8	p=0.735
	Ave (SD)	107.3±29.3	98.6±31.0		103.7±56.5	111.1±40.4		-3.7±41.1	4.0±50.5	
Ferritin	n	9	11	p=0.786	9	8	p=0.174	9	8	p=0.014
	Ave (SD)	29.6±20.2	32.3±23.1		19.2±15.5	34.0±26.4		-10.4±6.4	-1.5±6.7	
TCho	n	9	11	p=0.260	9	8	p=0.114	9	8	p=0.859
	Ave (SD)	168.1±20.4	155.4±27.1		176.4±24.2	158.3±19.8		8.3±11.6	7.3±13.1	
HDL	n	9	11	p=0.268	9	8	p=0.282	9	8	p=0.604
	Ave (SD)	59.6±10.1	55.0±7.7		62.0±9.0	56.9±10.0		2.4±3.2	1.5±4.2	
LDL	n	9	11	p=0.390	9	8	p=0.226	9	8	p=0.712
	Ave (SD)	99.4±17.9	90.6±25.2		100.2±22.2	88.4±15.5		0.8±9.0	2.9±13.8	

Test items		Before intervention		T test/ Wilcoxon	After intervention		T test/ Wilcoxon	After-Before		T test/ Wilcoxon
		Cocoa	Control		Cocoa	Control		Cocoa	Control	
TG	n	9	10	p=0.753	9	8	p=0.228	9	7	p=0.670
	Ave (SD)	86.6±54.3	80.2±30.3		93.2±50.7	69.1±20.0		6.7±47.8	-2.0±24.4	
TP	n	9	11	p=0.196	9	8	p=0.339	9	8	p=0.357
	Ave (SD)	7.57±0.39	7.32±0.43		7.59±0.49	7.39±0.32		0.02±0.28	0.14±0.21	
Alb	n	9	11	p=0.112	9	8	p=0.344	9	8	p=0.228
	Ave (SD)	4.77±0.22	4.57±0.28		4.71±0.29	4.59±0.22		-0.06±0.21	0.06±0.17	
AST	n	9	11	p=0.338	9	8	p=0.313	9	8	p=0.284
	Ave (SD)	17.2±2.7	18.6±3.5		18.3±3.3	21.5±8.4		1.1±1.3	2.9±4.6	
ALT	n	9	11	p=0.151	9	8	p=0.255	9	8	p=0.442
	Ave (SD)	10.0±2.5	12.5±4.3		11.0±2.4	13.3±5.1		1.0±1.7	2.0±3.4	
ALP	n	9	11	p=0.261	9	8	p=0.250	9	8	p=0.528
	Ave (SD)	193.1±44.5	233.5±95.9		187.8±43.4	233.1±104.4		-5.3±10.2	1.5±30.0	
BUN	n	9	11	p=0.681	9	8	p=0.113	9	8	p=0.199
	Ave (SD)	11.70±2.75	12.07±1.01		10.86±2.10	12.55±2.03		-0.84±2.49	0.70±2.21	
Crea	n	9	11	p=0.587	9	8	p=0.837	9	8	p=0.329
	Ave (SD)	0.494±0.042	0.507±0.058		0.500±0.048	0.505±0.050		0.006±0.017	-0.013±0.051	
UA	n	9	11	p=0.104	9	8	p=0.840	9	8	p=0.014
	Ave (SD)	4.37±0.67	5.09±1.11		4.51±0.65	4.58±0.62		0.14±0.29	-0.33±0.41	
HbA1c	n	9	11	p=0.994	9	8	p=0.177	9	8	p=0.729
	Ave (SD)	5.14±0.23	5.15±0.31		5.20±0.19	5.06±0.21		0.06±0.17	0.03±0.18	
25-OHVD	n	9	11	p=0.445	9	8	p=0.192	9	8	p=0.615
	Ave (SD)	17.92±6.71	19.89±4.53		14.17±4.89	17.65±5.64		-3.76±3.86	-2.79±3.91	
Zn	n	9	11	p=0.790	9	8	p=0.387	9	8	p=0.121
	Ave (SD)	97.4±11.7	99.3±17.3		102.4±13.7	96.8±12.5		5.0±8.2	-3.6±13.1	
Cu	n	9	11	p=0.485	9	8	p=0.586	9	8	p=0.643
	Ave (SD)	94.3±15.3	99.9±18.9		94.9±16.4	100.1±22.2		0.6±11.9	4.0±17.9	
DGLA	n	9	11	p=0.338	9	8	p=0.016	9	8	p=0.034
	Ave (SD)□	30.84±9.19	34.67±8.20		38.72±8.90	28.33±6.46		7.88±14.81	-7.95±12.97	
AA	n	9	11	p=0.407	9	8	p=0.647	9	8	p=0.430
	Ave (SD)	153.16±22.33	165.48±38.40		167.86±25.01	159.49±46.83		14.70±32.93	-4.38±61.54	
EPA	n	9	11	p=0.126	9	8	p=0.556	9	8	p=0.125
	Ave (SD)	29.60±28.51	15.04±9.19		20.43±12.53	24.41±14.76		-9.17±33.40	11.81±15.48	
DHA	n	9	11	p=0.054	9	8	p=0.744	9	8	p=0.230
	Ave (SD)	79.2±16.9	60.3±22.8		72.2±27.8	68.3±20.1		-6.9±34.2	11.4±24.9	
EPA/AA	n	9	11	p=0.130	9	8	p=0.420	9	8	p=0.137
	Ave (SD)	0.144±0.100	0.089±0.053		0.133±0.071	0.163±0.074		-0.011±0.159	0.088±0.082	
DHA/AA	n	9	11	p=0.005	9	8	p=0.559	9	8	p=0.021
	Ave (SD)	0.526±0.128	0.361±0.103		0.411±0.127	0.450±0.141		-0.114±0.193	0.108±0.157	
(EPA+DHA)/ AA	n	9	11	p=0.016	9	8	p=0.427	9	8	p=0.035
	Ave (SD)	0.668±0.223	0.449±0.146		0.522±0.179	0.600±0.214		-0.146±0.333	0.184±0.238	

Wilcoxon was used for GHQ 12 and for J-PSC 17.
T-test was used for all other test analysis.
Ave, Average; SD, Standard Deviation; BH, Body Height; BW, Body Weight; BMI, Body Mass Index; GHQ12, General Health Questionnaire 12; J-PSC17, Japanese version Pediatric Stress Checklist 17; WBC, White Blood Cell; RBC, Red Blood Cell; Hb Hemoglobin; Ht, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hematocrit; MCHC, Mean Corpuscular Hemoglobin Concentration; Plt, Platelet; Na, Sodium; Cl, Chloride; Ca, Calcium; P, Phosphorus; Mg, Magnesium; Fe, Serum Iron; Ferritin, Ferritin; TCho, Total Cholesterol; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; TG, Triglyceride; TP, Total Protein; Alb, Albumin; AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase; BUN, Blood Urea Nitrogen; Crea, Creatinine; UA, Uric Acid; HbA1c, Hemoglobin A1c; 25-OHVD, 25-hydroxy vitamin D; Zn, Zinc; Cu, Copper; DGLA, Dihomo-gamma Linoleic Acid; AA, Arachidonic Acid; EPA, Eicosapentaenoic Acid; DHA, Docosahexiaenoic Acid.

Discussion

Our study showed a significant decrease in the GHQ-12 score in 12-year-old children who consumed cocoa drinks daily for four weeks compared with those who did not consume any cocoa products. The high GHQ-12 score signifies higher mental health risk. The fact that the cocoa group scored lower on the GHQ-12 after four weeks of cocoa drink consumption may mean that drinking cocoa contributed to lowering the stress of 12-year-olds. A cross-sectional survey of 13,626 adults in the US in NHANES showed that consumption of chocolate, especially dark chocolate, may be associated with lower depressive symptoms [21]. Another study suggested that flavanol-rich cocoa is effective in counteracting mental stress-induced endothelial dysfunction and improving peripheral

blood flow during stress [22]. Martin et al. reviewed the effect of cocoa and cocoa products on cognitive performance in young adults, suggesting a beneficial effect of cocoa on neuroplasticity [23]. However, a systematic review published in 2021 on the health effects of chocolate and cocoa showed no significant difference between the chocolate/cocoa group and control group on parameters related to skin, blood pressure, lipid profile, cognitive function, anthropometry, blood glucose, and quality of life [24].

Our study showed a significant increase in serum DGLA levels in the cocoa group compared with the control group. A 21-year follow-up study from Finland in 2017 involving men in the general population concluded that serum DGLA was inversely associated with the risk of depression [25]. The results of the GHQ-12, which reflected the stress levels of the cocoa group in our study, showed that a decrease in stress may be associated with an increase in DGLA. DGLA is categorized as an n-6 polyunsaturated fatty acid (PUFA), often contrasted with n-3 PUFA as it is anti-inflammatory and n-6 PUFA is pro-inflammatory; however, both are essential fatty acids and necessary for maintaining health [26].

Among the blood test results, ferritin significantly lowered in the cocoa group than in the control group in the analysis of covariance (ANCOVA). It should be noted that the ferritin levels were low at baseline. All groups had ferritin levels of < 30 ng/mL. According to the World Health Organization guidelines, ferritin levels < 30 ng/mL are considered deficient [27]. Several previous studies have shown that iron deficiency is prevalent in Japan, which was also observed in the present study. We considered an association between ferritin levels and menstruation, but previous studies have shown no such association [28].

One limitation of this study was that the reason for the lower stress levels in the cocoa group may be partly due to improved parent-child interaction and communication during the process of preparing cocoa drinks together at home daily for four weeks. Future studies should attempt a safe placebo-controlled trial that does not include cocoa ingredients. Another limitation of this study was that we included only 12-year-olds, and the sample size was small. We chose the age 12 as a representative age group under stress in Japan, especially in Tokyo capital where majority of 12-year-olds face entrance examination for junior high schools [29,30]. Future studies with larger sample sizes should be conducted to ensure robust applicability to the general population. Another limitation was that a strict control was not feasible for the control group. We requested that the control group do not take cocoa or cocoa contained products and continue with their "usual food/drink intake" and with their "regular" activities and had all the participants fill in daily record of activities but there could have been stress-relieving activities among the control group which we could not catch.

Conclusion

Our study showed that consuming a cup of cocoa drink daily for one month improved the mental health status of early adolescents who were more susceptible to stress. Blood test results showed a significant increase in DGLA in the cocoa group compared to the control group. These results suggest that a decrease in stress in children consuming cocoa may be associated with an increase in DGLA.

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Clinical Trial Registry

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Declaration of conflicting interests

Inagaki, Yamamoto and Matsui are employees of Morinaga & Co., Ltd at the time of this study. Itoh received monetary compensation for the study design and operation. The staff members of Morinaga & Co., Ltd., were not involved in the collection, statistical analysis, or medical interpretation of the data.

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