Mediterranean Journal of Nutrition and Metabolism 9 (2016) 87–93
DOI:10.3233/MNM-160061
IOS Press

Original Article

Gene-lifestyle interaction: The role of SNPs in UCP2 -866G/A and UCP3 -55C/T on dietary intake and physical activity in Indonesian obese female adolescents

Harry Freitag Luglio[a,*], Dian Eurike[b], Emy Huriyati[a], Madarina Julia[c] and Rina Susilowati[b]
[a]*Department of Nutrition and Health, Faculty of Medicine, Universitas Gadjah Mada, Indonesia*
[b]*Department of Histology and Cell Biology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia*
[c]*Department of Child Health, Faculty of Medicine, Universitas Gadjah Mada, Indonesia*

Received 1 April 2016
Accepted 12 May 2016

Abstract.
BACKGROUND: Obesity is linked to high dietary intake and low physical activity. Studies showed that those factors were not only regulated by environment but also regulated by genetic variations. However, the relationship has less been understood in obese children and adolescents.
OBJECTIVE: The objective of this study was to examine the role of SNPs (single nucleotide polymorphisms) in uncoupling protein (UCP) 2 -866G/A and UCP3 -55C/T on dietary intake and physical activity in obese female adolescents.
METHODS: This is an observational study with cross sectional design. Respondents were obese female adolescents enrolled from obesity screening done in six junior high schools in Yogyakarta.
RESULTS: Seventy eight obese female adolescents joined this study. From 2 SNPs that have been analysed, we found that SNPs in UCP2 was associated with dietary intake and physical activity ($p = 0.02$ and $p = 0.02$, respectively). Interestingly, subjects with combination of UCP2 -866GG and UCP3 -55CC had slightly higher percent fat to total energy intake compared to those with UCP2 -866AA and UCP3 -55TT (mean difference = -3.8 ± 1.9; $p = 0.059$).
CONCLUSION: We concluded that SNPs on UCP2 was related to dietary intake and physical activity in Indonesian obese female adolescents.

Keywords: Dietary intake, *UCP2*, *UCP3*, obesity, physical activity, adolescents

*Corresponding author: Harry Freitag Luglio, Department of Nutrition and Health, Faculty of Medicine, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, Indonesia. Tel./Fax: +62 274 547775; E-mail: harryfreitag@ugm.ac.id.

ISSN 1973-798X/16/$35.00

Table 1
Primers and restriction enzymes used for PCR-RFLP

Genes	Primers (forward)	Primers (reverse)	Restriction enzymes	Incubation temperature and time
UCP2	5' – CAC GCT GCT TCT GCC AGG AC – 3'	5' – AGG CGT CAG GAG ATG GAC CG – 3'	MluI	Overnight at 37°C
UCP3	5'-GAG CTA TAT TAA AGC ACC CCG GGT CAA GAG GAC-3'	5'- TCT GCT GCT TCT GGC TTG GCA CTG GTC TTA TAC ACC C-3'	SmaI	25°C for 4 hours

1. Introduction

The prevalence of childhood obesity has increased dramatically during the last the decades. Obesity has been associated with increased risk of hypertension, cardiovascular diseases and type 2 diabetes mellitus [1]. High energy intake accompanied by low physical activity have been associated with the increasing risk of obesity [2–3]. There were evidences showing that eating pattern and physical activity were not purely controlled by environmental factors but also genetically inherited [4]. It has been estimated that genetic factors was responsible for 80% of variance in body mass index [5]. In this study, we were interested in investigating the role of genetic variations in UCP2 and UCP3. UCP2 and UCP3 are genes involved in many process in human metabolism and have been reported to be associated with risk of obesity [6, 7]. UCP2 and UCP3 encode uncoupling protein 2 and 3, family of mitochondrial transport proteins that promote oxidative phosphorylation from adenosine triphosphate (ATP) and release energy as heat [8].

Although some studies have supported the associations between UCP2 -866G/A and UCP3 -55C/T gene polymorphism with obesity [6, 7, 9], there are limited information regarding the role of those SNPs on dietary intake and physical activity in obese adolescents. In this study, we aimed to assess the role of single nucleotide polymorphisms (SNPs) of UCP2 -866G/A and UCP3 -55C/T genes on dietary intake and physical activity in obese female adolescents.

2. Methods

A total of 2120 female adolescents in Yogyakarta, Indonesia were screened for obesity based on the parameter of body mass index for age. Subject's obesity status was defined when body mass index (BMI) were beyond 95th percentile of 2000 Growth Reference Standard (WHO-Centre for Disease Control). From those who were screened, 136 of them were obese and only 78 who were agreed to participate in this study. The inclusion criteria were female, obese with age range between 13–15 years old and agreed to participate in this study. Those who did not have the menstrual cycle were excluded in this study. Ethical clearance was obtained from Ethical Commission of Medical and Health Research, Universitas Gadjah Mada, Yogyakarta.

Interviews were done by trained enumerators to collect data on dietary intake and physical activity. A 24 hours dietary recall was done in 6 non-consecutive days to assess dietary intake including total energy, fat and carbohydrate intake. Beside the absolute intake, we also showed the dietary intake as proportion fat and carbohydrate intake towards total energy intake (%) and dietary intake per kg body weight. Data from the recalls were then converted to Kcal or mg with the software Nutrisurvey (EBISpro, Germany). Data on habitual physical activity were obtained by 6 days non-consecutive physical activity recalls. The amount of energy spent in the physical activity was expressed in METs (metabolic equivalents of tasks).

Samples of DNA were extracted from 5 mL of peripheral blood by using salting out methods. Genotyping of those six genes were performed using PCR-RFLP with primers as shown in Table 1. The PCR conditions

Table 2
Characteristics of subjects

Characteristics	Mean ± SD
Age (years)	13.7 ± 0.9
Body weight (kg)	70.5 ± 8.3
Height (cm)	154 ± 5
Body mass index (kg/m^2)	29.7 ± 2.6
Waist circumference (cm)	89.2 ± 6.5
Hip circumference (cm)	104.9 ± 5.9
Dietary Intake	
Energy intake (kcal/day)	1141 ± 306
Fat intake (g/day)	41.9 ± 13.9
Carbohydrate intake (g/day)	151.2 ± 42.9
Energy intake (kcal/kg BW/day)	16.42 ± 5.09
Fat intake (g/kg BW/day)	0.60 ± 0.23
Carbohydrate intake (g/kg BW/day)	2.17 ± 0.68
Physical activity level (METS)	1931 ± 130.1

were initiated at 96°C for 5 minutes, followed by 36 cycles of reaction (denaturation for 30 seconds at 95°C, annealing for 30 seconds at 68°C, extension for 30 seconds at 72°C) and final extension for 7 minutes at 72°C. The fragments were resolved on 3% agarose gel for electrophoresis [10].

Allele frequency, Hardy Weinberg Equilibrium (HWE) and were calculated for both SNPs. In order to analyze the relationship between genetic variations and dietary intake and physical activity, one-way ANOVA with Tukey's Multiple Comparison Test was used. In addition, analysis using dominant model, recessive model, over dominant model and log additive models were used for confirmation on analysis. Students *t*-test and Mann Withney test were used to analyzed the difference in dietary intake and physical activity between combined allelic variations and reference variation UCP2 -866GG and UCP3 -55CC. All analysis were done using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

3. Results

We include 78 obese subjects who were between 13–15 years old. They had mean body weight of 70.5 ± 8.3 kg and mean BMI of 29.7 ± 2.6 kg/m^2 (Table 2). Hardy-Weinberg Equilibrium (HWE) analysis showed that both UCP2 -866G/A and UCP3 -55C/T gene polymorphism were inside the equilibrium ($p = 0.49$ and $p = 0.09$, respectively). Minor and major alleles were presented in the two genes analysed and both alleles were under the HWE.

Table 3 showed the association between SNPs, dietary intake and physical activity. The Table showed that SNP in UCP2-866G/A was associated with energy intake and carbohydrate intake ($p = 0.02$ and $p = 0.01$, respectively). This association is consistent even after the energy intake was divided with body weight (kcal/kg body weight/day and g/kg body weight/day, respectively). On the other hand, UCP3 -55C/T was not associated with dietary intake but when dietary intake was divided with body weight this association became significant. UCP3 -55C/T was associated with energy and fat intake per kg body weight ($p = 0.029$ and $p = 0.007$, respectively).

In addition to dietary intake, the role of several SNPs of UCP2 -866G/A and UCP3 -55C/T on physical activity were also evaluated (Table 3). Using one-way ANOVA, we found that physical activity level were different with subjects carrying SNPs on *UCP2*. Subjects on UCP2 -866G/G had higher physical activity than

Table 3
The relationship between genetic variation in UCP2 and UCP3, dietary intake and physical activity

Dietary intake	UCP2 (mean ± SD)				UCP3 (mean ± SD)			
	A/A ($n=15$)	G/A ($n=43$)	G/G ($n=20$)	*P*	T/T ($n=7$)	C/T ($n=44$)	C/C ($n=27$)	*P*
Energy (kcal/day)	1268.0 ± 206.8	1055.0 ± 327.8	1232.0 ± 270.0	0.02	1193.0 ± 314.8	1107.0 ± 324.9	1184.0 ± 275.4	0.53
Fat (g/day)	44.9 ± 9.5	38.5 ± 15.5	46.9 ± 11.5	0.05	40.5 ± 11.9	40.3 ± 15.1	44.9 ± 12.2	0.38
Carbohydrate (g/day)	172.4 ± 36.7	139.1 ± 43.5	161.4 ± 38.7	0.01	165.0 ± 49.5	146.9 ± 44.7	154.8 ± 38.6	0.51
Energy (kcal/kg BW/ day)	17.2 ± 2.7	15.35 ± 5.90	18.16 ± 4.08	0.007	15.96 ± 4.44	15.67 ± 5.65	17.77 ± 4.07	0.029
Fat (g/ kg BW/ day)	0.60 ± 0.11	0.56 ± 0.28	0.69 ± 0.17	0.122	0.54 ± 0.16	0.57 ± 0.26	0.67 ± 0.18	0.007
Carbohydrate (g/ kg BW/ day)	2.34 ± 0.52	2.02 ± 0.74	2.38 ± 0.60	0.013	2.22 ± 0.72	2.07 ± 0.73	2.32 ± 0.58	0.110
Physical activity (METS)	1850 ± 106	1945 ± 129	1962 ± 129	0.02	1921 ± 90	1925 ± 142	1944 ± 121	0.83

those in other genotypes ($p=0{,}02$). The relationship between UCP2 -866G/A and carbohydrate intake as well as physical activity were confirmed in recessive model. However, that association was not seen in UCP3 -55C/T gene polymorphism.

In order to examine the influence of gene-gene interaction, the relationship between allelic combination of SNPs in UCP2 -866G/A and UCP3 -55C/T and dietary intake and physical activity were examined. We observed no statistically significant association between combination of UCP2 -866G/A and UCP3 -55C/T on dietary intake and physical activity ($p>0{,}05$). Interestingly, subjects with UCP2 -866GG and UCP3 -55CC were more likely to have higher percent dietary fat to total energy intake compared to those with UCP2 -866AA and UCP3 -55TT (Table 4). Although there was a trend, the association of combined UCP2-866AA and UCP3-55TT genotype with percent dietary fat (Table 3) did not reach a statistical significance ($p=0.059$), and the number of subjects in this subgroup was very small ($n=6$).

4. Discussion

In order to evaluate the influence of genetic factors on dietary habits and physical activity in obese female adolescents, SNPs from two genes *UCP2* and *UCP3* were analyzed. From those SNPs, we found that SNPs in UCP2 -866G/A was associated with dietary intake and physical activity. UCP3 -55C/T was associated with dietary intake after corrected for body weight. The role of combined gene polymorphisms on dietary intake and physical activity was also investigated in this study. We found that subjects with UCP2 -866AA and UCP3 -55TT gene polymorphism had lower percent fat intake for total energy intake compared to dominant alleles (GG and CC genotypes).

In this study, we found SNP on UCP2 gene was related to total energy and carbohydrate intake which was not seen in a study carried out/conducted by Damcott et al. [11]. They suggested that SNP on UCP3 but not UCP2 influenced energy intake while this relationship was not seen in this study. In our study, the role of UCP3 on intake were only significant after body weight was corrected by dividing energy intake with kg body weight. We assumed that the difference in our study than those done by Damcott et al. [11] was due to differences in age and ethnicity. In their study they used adult Caucasian subjects while in our study we used Asian female adolescents.

Obesity is associated with increased energy intake and reduced physical activity. Therefore genetic polymorphisms that increased dietary intake also increased risk for obesity and vice versa. The interaction between UCP2-866G/A genotype and lifestyle has been investigated in other studies with conflicting results. A study

Table 4
The combination of UCP2 and UCP3 gene polymorphisms on %fat and %carbohydrate to total energy intake

	CC	CT	TT
GG	($n = \mathbf{19}$)	($n = \mathbf{1}$)	N/A
	Energy/kg body weight/day	**Energy/kg body weight/day**	
	mean = 18.2 ± 4.2	mean = 17.4	
	mean diff. = N/A (ref.)	mean diff. = N/A	
	p = N/A (ref.)	p = N/A	
	% fat intake	**% fat intake**	
	mean = 34.1 ± 4.4	mean = 36.2	
	mean diff. = 0 (ref.)	mean diff. = N/A	
	p = N/A (ref.)	p = N/A	
	% carbohydrate intake	**% carbohydrate intake**	
	mean = 52.8 ± 5.1	mean = 48.1	
	mean diff. = 0 (ref.)	mean diff. = N/A	
	p = N/A (ref.)	p = N/A	
GA	($n = 6$)	($n = 36$)	($n = 1$)
	Energy/kg body weight/day	**Energy/kg body weight/day**	**Energy/kg body weight/day**
	mean = 15.8 ± 4.0	mean = 15.5 ± 6.2	mean = 8.2
	mean diff. = –2.4 ± 1.9	mean diff. = 2.7 ± 1.6	mean diff. = N/A
	p = 0.238	p = 0.089	p = N/A
	% fat intake	**% fat intake**	**% fat intake**
	mean = 33.2 ± 5.9	mean = 32.3 ± 3.6	mean = 31.84
	mean diff. = –0.9 ± 2.2	mean diff. = –1.8 ± 1.6	mean diff. = N/A
	p = 0.694	p = 0.267	p = N/A
	% carbohydrate intake	**% carbohydrate intake**	**% carbohydrate intake**
	mean = 51.4 ± 5.1	mean = 53.5 ± 6.7	mean = 47.2
	mean diff. = –1.4 ± 2.4	mean diff. = 0.7 ± 1.8	mean diff. = N/A
	p = 0.554	p = 0.697	p = N/A
AA	($n = 2$)	($n = 7$)	($n = 6$)
	Energy/kg body weight/day	**Energy/kg body weight/day**	**Energy/kg body weight/day**
	mean = 19.4 ± 2.2	mean = 16.5 ± 2.4	mean = 17.3 ± 3.1
	mean diff. = 1.2 ± 3.0	mean diff. = 1.7 ± 1.7	mean diff. = 0.9 ± 1.9
	p = 0.702	p = 0.313	p = 0.614
	% fat intake	**% fat intake**	**% fat intake**
	mean = 34.3 ± 2.6	mean = 32.7 ± 7.5	mean = 30.3 ± 3.1
	mean diff. = –0.18 ± 3.2	mean diff. = –1.4 ± 2.4	Mean diff. =–3.8 ± 1.9
	p = 0.957	p = 0.566	p = 0.059
	% carbohydrate intake	**% carbohydrate intake**	**% carbohydrate intake**
	mean = 53.1 ± 3.8	mean = 52.8 ± 8.1	mean = 56.1 ± 4.6
	mean diff. = 0.3 ± 3.7	mean diff. = 0.04 ± 2.6	mean diff. = 3.3 ± 2.3
	p = 0.927	p = 0.989	p = 0.172

done by Jun et al. [9], which was conducted in a large sample of Korean children ($n = 737$) and adults ($n = 732$), showed a protective effect of UCP2-866A and UCP3-55T against the risk of overweight in children. Korean children carrying the combined non-UCP2-866GG and non-UCP3-55CC genotype had lower BMI and lower obesity risk. In our study, subjects with non-UCP2-866GG or non-UCP3-55CC genotypes had lower energy intake per kg body weight and this was allign to those found in Korean children.

In our previous report, we showed that UCP2 is interacted with lifestyle factors such as dietary fat intake in order to induce obesity. UCP2 alone was not associated with obesity. However, when subjects were separated in high fat diet group, UCP2-866G>A polymorphism were associated with protection towards obesity [12]. Although SNPs in UCP family was associated with body composition, dietary intake and energy metabolism, there is no clear mechanism of this relationship. It was speculated that differences in caloric intake between those with UCP2 and UCP3 gene polimorphisms were due to compensation on metabolic rate [11].

The role of UCP2 gene polymorphism on physical activity was initially seen in a metabolic chamber. Astrup et al. [13] analyzed the effect of v/v 55 polymorphism in the UCP2 gene on the metabolic rate of Danish Caucasian subjects and found that the polymorphism was related to differences in spontaneous physical activity. One of the explanation of this effect is that subjects with val/val genotype have higher energy efficiency than subjects with ala/ala genotype [14]. Although the gene that is reported in our study is similar with those analyzed by them [13, 14], the SNP location were different. It might that UCP2 polymorphism affected energy efficiency in obese adolescent girls of our study, thus influenced their physical activity level. In our previous report, we showed that UCP2 is interacted with dietary fat intake in order to induce obesity. This effect was not seen when dietary fat was not included in the analysis [12].

Though the association did not reach the statistical significance, adolescents carrying the combined UCP2-866AA and UCP3-TT (Table 3) showed a trend towards a lower fat intake. This association has not been previously reported in Asian adolescents. In our obese female adolescent subjects, we showed that subjects with UCP2 -866GG and UCP3 -55CC had higher dietary fat intake compared to UCP2 -866GA/AA and UCP3 -55CT/TT. This findings indicated the mechanism on how the combination of UCP2 and UCP gene polymorphism was associated with overweight and obesity. The effect of combination of both genes on obesity were also seen in Korean children [9].

There were some limitations in this study. Number of subjects in this study is limited and subjects is limited to female obese adolescents therefore cannot be interpreted to the general adolescents population in Indonesia. The dietary record in this study was 24 hours dietary recall which is potential for under-reporting. We tried to maximize the accountability of the method by using the recording period into 6 nonconsecutive days.

We concluded that SNPs on UCP2 -866G/A was associated to dietary intake and physical activity in Indonesian obese female adolescents. The combination of UCP2 -866G/A and UCP3 -55C/T was associated with dietary fat intake in our subjects. This data confirmed on how SNPs in UCP2 -866G/A and UCP3 -55C/T were associated with overweight that was showed by previous studies. This initial data on gene-lifestyle association is essential to be used as the predictors of obesity related behavior in children and adolescents. Further study is necessary to investigate the effect of those genes on dietary intake and physical activity at the broader population. Studies to investigate the role of those SNPs on successfulness of weight loss in obese adolescents are warranted.

References

[1] Daniels SR, Arnett DK, Eckel RH, et al. Overweight in children and adolescents: Pathophysiology, consequences, prevention, and treatment. Circulation. 2005;111:1999-2012. doi:10.1161/01.CIR.0000161369.71722.10

[2] Dietz WH. Overweight in childhood and adolescence. N Engl J Med. 2004;350:855-7. doi:10.1056/NEJMp048008

[3] Martinez JA. Body-weight regulation: Causes of obesity. Proc Nutr Soc. 2000;59:337-45. doi:10.1017/S0029665100000380

[4] De Castro JM. Genetic influences on daily intake and meal patterns of humans. Physiol Behav. 1993;53:777-82. doi:10.1016/0031-9384(93)90188-L

[5] Phillips C. Nutrigenetics and metabolic disease: Current status and implication for personalized nutrition. Nutrients. 2013;5(1):32-57.

[6] Oktavianthi S, Trimarsanto H, Febinia CA, et al. Uncoupling protein 2 gene polymorphisms are associated with obesity. Cardiovasc Diabetol. 2012;11:41. doi:10.1186/1475-2840-11-41

[7] Salopuro T, Pulkkinen L, Lindström J, et al. Variation in the UCP2 and UCP3 genes associates with abdominal obesity and serum lipids: The Finnish Diabetes Prevention Study. BMC Med Genet. 2009;10:94. doi:10.1186/1471-2350-10-94

[8] Klaus S, Casteilla L, Bouillaud F, Ricquier D. The uncoupling protein UCP: A membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. Int J Biochem. 1991;23(9):791-801.

[9] Jun HS, Kim IK, Lee HJ, et al. Effects of UCP2 and UCP3 variants on the manifestation of overweight in Korean children. Obesity (Silver Spring). 2009;17(2):355-62. doi:10.1038/oby.2008.531

[10] Eurike D, Muhammad HFL, Susilowati R, Julia M. UCP3 gene polymorphism and insulin resistance in obese female adolescents. Paediatr Indones. 2012;52:152-56.

[11] Damcott CM, Feingold E, Moffett SP, et al. Genetic variation in uncoupling protein 3 is associated with dietary intake and body composition in females. Metabolism. 2004;53:458-64. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15045692.

[12] Huriyati E, Luglio HF, Ratrikaningtyas PD, Tsani AFA, Sadewa AH, Juffrie M. Dyslipidemia, insulin resistance and dietary fat intake in obese and normal weight adolescents: The role of uncoupling protein 2 -866G/A gene polymorphism. Int J Mol Epidemiol Genet. 2016;7(1):67-73.

[13] Astrup A, Toubro S, Dalgaard LT, Urhammer SA, Sorensen TI, Pedersen O. Impact of the v/v 55 polymorphism of the uncoupling protein 2 gene on 24-h energy expenditure and substrate oxidation. Int J Obes Relat Metab Disord. 1999;23:1030-4. doi:10.1038/sj.ijo.0801040

[14] Buemann B, Schierning B, Toubro S, et al. The association between the val/ala-55 polymorphism of the uncoupling protein 2 gene and exercise efficiency. Int J Obes Relat Metab Disord. 2001;25:467-71. doi:10.1038/sj.ijo.0801564