The role of uncoupling protein 2 and 3 genes polymorphism and energy expenditure in obese Indonesian children

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Abstract

Aim: Uncoupling protein (UCP) genes, which may contribute to energy metabolism in mitochondria, may be involved in the pathogenesis of obesity. We analyzed the differences in energy expenditure between single nucleotide polymorphisms (SNPs) *UCP3-55C/T*, *UCP3 Y210Y*, and *UCP2 A55V* among Indonesian children.

Methods: The study included 76 schoolchildren (36 obese and 40 healthy; mean age, 12.8 years) in Semarang, Indonesia. Body composition was measured by bioelectrical impedance analysis; resting energy expenditure (REE) by indirect calorimetry; physical activity by uniaxial accelerometer; and total energy expenditure (TEE) by the equations extrapolated from REE and physical activity. *UCP3-55C/T*, *UCP3 Y210Y*, and *UCP2 A55V* were examined by restriction length fragment polymorphism analysis.

Results: The TEE of the subjects with the T/T genotype at *UCP3-55C/T* after adjusting for fat-free mass (63.2±7.2 kcal/kg/day) and T/T at *UCP2 A55V* (62.8±5.6 kcal/kg/day) was lower than that of the subjects with the C/C and C/T genotypes ($p<0.05$). The REE of the subjects with these T/T genotypes tended to be lower than that of the subjects with C/C and C/T ($p\geq0.05$). No significant differences in REE or TEE were found between the *UCP3 Y210Y* genotypes.

Conclusions: The subjects with the T/T genotypes of *UCP3-55C/T* or *UCP2 A55V* had lower TEE than those with other genotypes.

Keywords: children; energy expenditure; Indonesia; obesity; UCP genes.

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Introduction

Obesity is considered a global epidemic because its prevalence and severity in adults and children is increasing at an alarming rate worldwide (1, 2). Differences in energy metabolism may play a role in long-term body weight regulation and the pathogenesis of human obesity. The largest component of energy expenditure is resting energy expenditure (REE), which is the energy expended to maintain the basic physiological functions of the body. Meanwhile, physical activity is responsible for 25% to 31% of total energy expenditure (TEE) and is a key component of energy balance (3–5). The observed variations in body mass index (BMI) and fat mass may be genetically determined. Moreover, an increasing number of studies are reporting associations between DNA sequence variation in specific genes and obesity phenotypes (6, 7). Susceptibility to obesity is partly determined by genetic factors, but an obesity-promoting environment is typically necessary for their phenotypic expression. Such a genetically mediated susceptibility to environmental exposure is referred to as gene-environment interaction (6, 8). However, many genes are regulated in obesity, called polygenic genes (7, 9).

Uncoupling proteins (UCPs) are a family of mitochondrial transporters known to uncouple oxidative phosphorylation via proton leakage from the inner mitochondrial membrane. These mitochondrial proteins are implicated as potential regulators of thermoregulation and energy metabolism. More than five types of UCPs have been identified to date. *UCP1* is expressed exclusively in brown adipose

tissue and is responsible for thermogenesis in mammalian neonates and rodents; *UCP2* is expressed in almost all mammalian tissues; *UCP3* is predominantly expressed in mammalian skeletal muscle and brown adipose tissue (9–11). In humans, *UCP2* and *UCP3* form a cluster on chromosome 11q13. The chromosomal location and tissue distribution patterns of *UCP2* and *UCP3* suggest that polymorphisms of these UCPs contribute to metabolic disorders via their major effects on energy metabolism (12, 13).

A number of polymorphisms have been identified in the *UCP2* and *UCP3* genes, and their correlations with obesity-related phenotypes and body weight regulation have been evaluated in adult subjects. In particular, the *–55C/T* polymorphism of the *UCP3* gene and the *A55V* polymorphism of the *UCP2* gene are responsible for changes in UCP mRNA levels (14, 15), adult REE (16, 17), and weight gain. In addition, body fat percentage is genetically linked to the *UCP2/UCP3* locus (18) and it is associated with oxidation rate and respiratory quotient (RQ) (19, 20) in populations with a marked susceptibility to obesity (21). The RQ of subjects with the homozygous *v/v* at *A55V* is higher than that of A/A and A/V subjects (20) and influences gross exercise efficiency (22). Other studies have failed to find relationships between *UCP2* and *UCP3*, and energy expenditure (18, 23, 24). Although a small number of studies have examined the effects of UCP variants on obesity in young children, some also report associations between *UCP2* and *UCP3*, and childhood obesity (25, 26). Such discrepancies in the literature may be attributable to differences in study design (e.g., population based vs. hospital based), selection and ascertainment schemes, sample sizes, or statistical analysis strategies (27). However, there is no study on the associations between energy expenditure and *UCP2* and *UCP3* involving Indonesian children. Therefore, we examined the roles of the polymorphisms of *UCP3-55C/T*, *UCP3 Y210Y*, and *UCP2 A55V* in energy expenditure in obese and non-obese Indonesian children.

Subjects and methods

A total of 76 subjects were included in this study. The study was conducted at a junior high school in Semarang City, Indonesia, from 2005 to 2007. The obese children were 11–15 years old, and lean age-matched counterparts were recruited as controls. The study protocol was approved by the regional Ethical Committee, and informed consent was obtained from the parents of all the children.

We evaluated all of the students at the school and selected obese and lean subjects by simple random sampling. Body composition was measured by bioelectrical impedance analysis (BIA) Tanita TBF-310 (Tanita Corp., Tokyo, Japan), which included weight and body fat, fat mass, and fat-free mass percentages. Studies have shown that differences of non-invasive methods to estimate fat mass such as dual-energy X-ray absorptiometry (DEXA), isotope dilution, BIA, and skinfold thickness had variations in marked body fat in children (28, 29). Considering the availability of the facility and the advantages of BIA for use in a large-scale study (28), we decided to measure the fat mass and calculate the fat-free mass by BIA. Height was measured by a mounted stadiometer. BMI was calculated as body weight (kg) divided by height (m) squared. Currently, there are no guidelines for classifying weight for children under 18 years in Indonesia or Indonesian standards for tracking growth in children, therefore, we used the CDC 2000 BMI growth chart. The cut-offs were as follows: children above the 95th percentile were classified as obese and those between the 5th and 85th percentiles were classified as healthy (i.e., controls).

Energy expenditure

REE was measured using an indirect calorimetry system (AR-1, Arco System, Chiba, Japan). REE was measured in the morning after 10–12 h of fasting in a temperature-controlled room over a single 5-min period. The energy values were measured using the Douglas bag technique. The resting metabolism rate was calculated as the mean of two values for lying down and sitting (30). Physical activity was measured using a 1-axial KenzLifecorder accelerometer. For each participant, a portable activity monitor based on a uniaxial accelerometry sensor (Lifecorder, Suzuken, Nagoya, Japan) was attached at the waist, and acceleration was recorded for 7 consecutive days. As it was small and light (62×46×26 mm, 40 g), the subjects were not disturbed by the machine (31). The accelerometer provided activity data every 2-min, as well as the step data. TEE was measured using equations combined from indirect calorimetry and accelerometer (Σ METs×min×measured REE). The sum of metabolic equivalents (Σ METs) was extrapolated from the accelerometer data according to a previous study (32).

Genotyping

DNA was extracted from blood samples. Genotyping was done using restriction fragment length polymorphism analysis at 3 polymorphic sites: *UCP3-55C/T*, *UCP3 Y210Y*, and *UCP2 A55V*. The DNA was amplified by polymerase chain reaction (PCR) with specific primers. The forward and reverse primers for the *UCP3-55C/T* sites were 5′-cct ccc ctc tca cct cac tg-3′ and 5′-ggc act ggt ctt ata ccc ac-3′, respectively. The 115-bp product was digested with *Bse*DI. The C allele gave 94- and 21-bp fragments, whereas the T/T allele gave a 115-bp fragment. The forward and reverse primers for the silent mutation *Y210Y* (TAC→TAT) variant were 5′-tca agg aga agc tgc tgg agt-3′ and 5′-tac tag gca ctg ctt ctc tct ctg-3′, respectively. The *Y210*(C) allele gave 110- and 20-bp fragments after being digested by the *Rsa*I enzyme, and the *Y210*(T) allele gave a 130-bp fragment. The forward and reverse primers for *UCP2 A55V* (GCC→GTC) were 5′-ctg gag tct cga tgg tgt ctac-3′ and 5′-cac cgc ggt act ggg cgt tg-3′, respectively. The 198-bp product was digested by the *Hinc*II restriction enzyme and gave 180- and 18-bp fragments for the T allele and a 198-bp fragment for the C allele. DNA fragments were resolved on a 4% agarose gel. The DNA extraction was done in the Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, and the genotype analysis was done in the Laboratory of Human Biology and Genetics, Department

of Biological Sciences, Graduate School of Sciences, University of Tokyo, Tokyo. Statistical analysis was performed using SPSS 11.0 (Chicago, IL, USA), and linkage disequilibrium was analyzed using the Haploview program (Broad Institute of MIT and Harvard; http://www.broad.mit.edu/haploview/haploview).

Results

Seventy-six children, including 36 obese (25 boys and 11 girls) and 40 lean children (16 boys and 24 girls), with a mean age of 12.8 years, participated in this study. The characteristics of the study population are shown in Table 1. Both sexes of the obese subjects had higher REE, however, after adjusting for body weight, the obese subjects had lower REE/body weight. All the children were genotyped. There were 24, 42, and 10 C/C, C/T, and T/T, respectively, on the *-55C/T* promoter region of *UCP3*. For the *UCP3 Y210Y* genotyped at exon 5; 29, 41, and 56 children were C/C, C/T, and T/T, respectively. For *UCP2* exon 4 *A55V*; 28, 39, and nine children were C/C, C/T, and T/T, respectively. All the polymorphisms at the promoter region and exons were in linkage disequilibrium (data not shown).

Table 2 and Figure 1 showed that the REE was lower in the subjects with the T/T genotypes of *UCP3-55C/T* and *UCP2 A55V*, although the difference was not significant. Meanwhile, the results regarding the silent mutation of exon 5 *UCP3* were inconsistent. The subjects with C/T had lower REE and TEE than those with other genotypes. The TEE, which was calculated on the basis of the REE and physical activity measured by an accelerometer, showed that the TEE of the T/T *UCP2 A55V* subjects was significantly lower (2289±418 kcal/day) than those with the C/C and C/T genotypes. After adjusting for fat-free mass, the results regarding TEE/ffm/day were also similar: the subjects with T/T (62.8±5.6 kcal/kg/day) had lower TEE/ffm/day than those with C/C (68.9±9.3 kcal/kg/day) and C/T (74.5±19.8 kcal/kg/day).

The REE of the obese subjects with the T/T genotype was lower than those of the obese subjects with the C/C and C/T genotypes, although the difference was not significant. In contrast, the obese children with the C/T genotype of *Y210Y UCP3* had the lowest TEE among all the genotypes, but the difference was also not significant. Similar results were found among the healthy children: the TEE of the subjects with the C/T *Y210Y* genotype was the lowest. The TEE/ffm/day of the subjects with T/T on promoter *UCP3-55C/T* and *UCP2 A55V* was the lowest among all the genotypes; the difference was significant in the healthy children. These results indicate that in addition to REE, physical activity contributes to the energy expenditure in healthy children (Table 3).

Discussion

The subjects with a T/T genotype at the *UCP3-55C/T* and *UCP2 A55V* polymorphisms had lower TEE than those with the C/C and C/T genotypes. However, we did not find any association between the T/T genotypes and REE, although the subjects with the T/T genotypes had lower REE than those with other genotypes. The association between the *UCP3-55C/T* polymorphism and REE in various populations raises the hypothesis that this polymorphism modifies gene expression and therefore modulates energy homeostasis (33). The mitochondrial protein UCP3 is mainly expressed in skeletal muscle and possesses uncoupling activity (34). Pima Indians, who carry the –55 T allele, have significantly higher *UCP3* mRNA concentrations than those with the –55 C/C allele. This indicates that the –55 T allele can increase *UCP3* mRNA

Table 1 Subject characteristics.

	Boys		Girls	
	Obese	Healthy	Obese	Healthy
n	25	16	11	24
Age, years	12.8±0.3	12.9±0.4	12.6±0.5	12.8±0.4
Weight, kg	66.2±7.7	44.8±7.1	68.1±10.9	42.9±6.1
Height, cm	155.0±7.1	156.5±8.0	152.4±7.1	151.1±6.1
BMI, kg/m²	27.5±2.4	18.2±1.7	29.2±2.9	18.7±1.9
% fat	32.3±10.9	16.4±4.7	38.3±4.4	21.5±4.6
REE/day, kcal	2015±295	1717±298	1866±292	1607±385
REE/weight/day, kcal/kg	30.6±3.5	38.9±7.9	27.5±2.2	37.9±9.8
TEE/day, kcal	3131±486	2558±482	2832±547	2427±609
TEE/weight/day, kcal	47.5±6.3	58.4±15.1	41.7±5.3	57.3±15.4

BMI, body mass index; REE, resting energy expenditure; TEE, total energy expenditure. The values are mean±SD.

Table 2 Genotype distribution and energy expenditure parameters.

SNPs	Variables	C/C	C/T	T/T	p-Value
UCP3 -55C/T	n	24	42	10	
	REE/day, kcal	1836±302	1820±418	1641±188	0.140
	REE/weight/day, kcal/kg	32.6±5.9	35.5±9.6	32.5±5.3	0.267
	REE/ffm/day, kcal/kg	46.2±7.8	47.9±11.1	42.4±4.2	0.167
	TEE/day, kcal	2754±498	2809±684	2455±413	0.262
	TEE/weight/day, kcal/kg	48.9±8.3	54.8±15.9	48.2±7.0	0.146
	TEE/ffm/day, kcal/kg	69.3±12.2	74.0±18.3	63.2±7.2	0.044[a]
UCP3 Y210Y	n	29	41	6	
	REE/day, kcal	1864±409	1766±344	1742±238	0.395
	REE/weight/day, kcal/kg	35.0±11.8	33.6±4.9	34.1±4.6	0.637
	REE/ffm/day, kcal/kg	49.3±13.9	45.2±5.2	44.4±1.7	0.790
	TEE/day, kcal	2867±697	2650±545	2803±475	0.340
	TEE/weight/day, kcal/kg	53.8±18.9	50.4±8.3	54.6±5.3	0.423
	TEE/ffm/day, kcal/kg	75.6±22.4	67.8±9.4	71.3±4.5	0.329
UCP2 A55V	n	28	39	9	
	REE/day, kcal	1848±391	1822±359	1571±198	0.058
	REE/weight/day, kcal/kg	32.8±5.7	35.0±10.1	34.6±4.5	0.385
	REE/ffm/day, kcal/kg	45.4±6.7	48.4±11.8	43.3±1.4	0.218
	TEE/day, kcal	2816±632	2799±588	2289±418	0.019[a]
	TEE/weight/day, kcal/kg	50.0±7.9	54.0±16.8	50.1±6.9	0.812
	TEE/ffm/day, kcal/kg	68.9±9.3	74.5±19.8	62.8±5.6	0.021[a]

BMI, body mass index; REE, resting energy expenditure; REE/ffm/day, REE/fat-free mass/day; TEE, total energy expenditure; TEE/ffm/day, TEE/fat-free mass/day. The values are mean±SD. Kruskal-Wallis test (non-parametric data).
[a]Significantly different from T/T at $p<0.05$.

expression more than the –55 C allele (15). In contrast, in the present study, the subjects carrying the –55 T/T genotype had lower TEE than those with C/C and C/T. A study on Danish men assessed the role of physical activity as an effect modifier in the *UCP3-55C/T* polymorphism (23), although there was no evidence of interaction between the UCP variants and physical activity. However, a study in the Spanish population found that the *UCP3-55C/T* polymorphism is associated with a lower risk of obesity when recreational energy expenditure was taken into consideration. The possible explanation for this finding is similar to that of a previous study: decreasing *UCP3* expression in the *–55C/T* polymorphism increases energy expenditure associated with physical activity (27). As a result of the contribution of energy from physical activity to the TEE, we assumed that physical activity plays a role in a variety of *UCP3-55C/T* polymorphisms.

The *UCP3 Y210Y* polymorphism was not associated with energy expenditure. The genotype frequency of the *UCP3 Y210Y* polymorphism in the present study was comparable between the obese and healthy subjects, concordant with the Quebec Family study (35) and Heritage

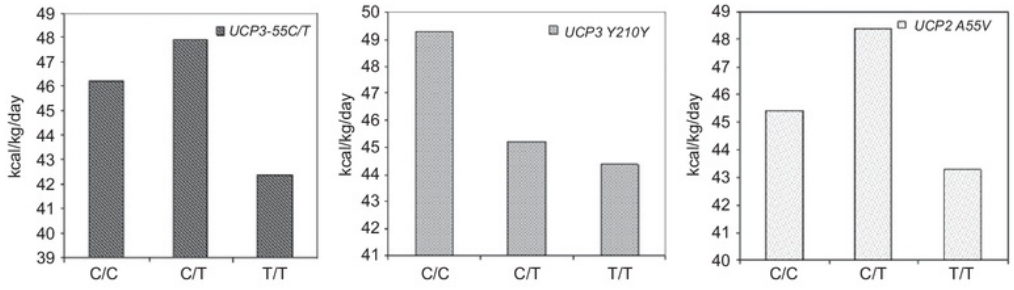

Figure 1 Resting energy expenditure (REE) after adjusting for fat-free mass. *Significantly different from T/T at the *UCP3-55C/T* and *UCP2 A55V* at $p<0.05$.

Table 3 Genotype distribution and energy expenditure parameters in obese and normal subjects

SNPs		Obese			Non-obese		
		C/C	C/T	T/T	C/C	C/T	T/T
UCP3 -55C/T	n	13	19	4	11	23	6
	REE/day, kcal	2013±228	1967±362	1829±91	1626±242	1698±429	1515±107
	REE/weight/day, kcal/kg	29.3±3.9	30.0±3.6	28.0±3.5	36.3±6.2	39.9±10.8	35.5±4.0
	REE/ffm/day, kcal/kg	47.1±8.4	45.7±6.3	40.9±6.1	45.2±7.3	49.8±13.7	43.5±2.6
	TEE/day, kcal	3060±417	3066±618	2848±266	2393±311	2596±675	2193±240
	TEE/weight/day, kcal/kg	44.8±5.9	46.8±7.0	43.7±7.13	53.7±8.3	61.3±18.2	51.2±5.5
	TEE/ffm/day, kcal/kg	71.6±14.4	70.8±9.6	63.6±9.7	66.5±8.8	76.5±23.2	62.9±6.2[a]
UCP3 Y210Y	n	15	18	3	14	23	3
	REE/day, kcal	1999±263	1952±350	1926±123	1720±493	1621±264	1557±157
	REE/weight/day, kcal/kg	29.3±3.3	29.7±3.2	30.7±3.3	41.1±14.4	36.6±3.6	37.6±2.5
	REE/ffm/day, kcal/kg	47.7±8.4	44.4±6.3	43.3±0.4	51.0±18.3	45.8±4.2	45.5±1.8
	TEE/day, kcal	3099±493	2966±568	3187±345	2619±811	2402±381	2419±65
	TEE/weight/day, kcal/kg	45.5±7.4	45.1±6.1	50.5±3.7	62.6±23.3	54.6±7.5	58.6±2.9
	TEE/ffm/day, kcal/kg	73.7±13.6	67.3±10.0	71.5±3.9	77.7±29.5	68.2±9.2	71.1±6.0
UCP2 A55V	n	15	19	2	13	20	7
	REE/day, kcal	2071±380	1905±208	1824±192	1590±206	1743±451	1499±137
	REE/weight/day, kcal/kg	30.0±3.6	29.4±3.3	28.8±5.4	36.1±6.0	40.4±11.4	36.2±2.7
	REE/ffm/day, kcal/kg	45.7±6.6	45.9±7.9	43.1±0.1	45.1±7.1	50.7±14.4	43.3±1.6
	TEE/day, kcal	3191±619	2947±404	2789±632	2383±268	2659±704	2146±242[a]
	TEE/weight/day, kcal/kg	46.3±6.9	45.4±0.59	44.2±13.5	54.0±7.1	62.2±19.7	51.8±4.2
	TEE/ffm/day, kcal/kg	70.3±10.7	70.9±12.8	65.5±8.2	67.4±7.6	77.9±24.6	62.0±5.3[a]

BMI, body mass index; REE, resting energy expenditure; REE/ffm/day, REE/fat-free mass/day; TEE, total energy expenditure; TEE/ffm/day, TEE/fat-free mass/day. The values are mean±SD. Kruskal-Wallis test (non-parametric data).
[a]Significantly different from T/T at $p<0.05$.

cohort study (36). Both studies found that the *UCP3 Y210Y* polymorphism is not associated with adiposity (35) and did not contribute to adiposity after 20 weeks of endurance training (36). *UCP Y210Y* is a silent mutation that is not expected to alter the function of the UCP3 protein, as found in the present study. In contrast, a previous study on racial differences regarding the relationship between uncoupling protein genes and REE found that the REE values of African-American women with the C/C genotype of *UCP3 Y210Y* are significantly lower than those of Caucasian women with the same genotype (21). No variation in REE was observed with respect to *–55C/T*, but the REE was significantly lower in subjects with the C/C genotype of *Y210Y* than in those with the T/T genotype (23). Thus, the inconsistencies among some studies indicate that the silent mutation may only play a role in specific populations.

The *UCP2 A55V* in exon 4 is one of the most commonly studied variants. This variant is reported to be associated with the sleeping metabolic rate (17), energy expenditure adjusted for fat-free mass, fat mass, spontaneous physical activity (20), and exercise efficiency while bicycling (22). Astrup et al. (20) report that 24-h energy expenditure adjusted for fat-free mass, fat mass, and spontaneous physical activity is lower in subjects with the T/T genotype than those with other genotypes. Similarly, the 24-h RQ adjusted for energy balance, age, sex, and spontaneous physical activity is higher in subjects with T/T than those with C/C and C/T genotypes. Therefore, subjects with the *UCP2* T/T genotype exhibit enhanced metabolic efficiency and lower fat oxidation compared with those with other genotypes. The present result shows that the subjects with the T/T genotype had lower REE and TEE than those with the C/C and C/T genotypes, although only TEE was significantly lower (20). This indicates that physical activity contributes to TEE, which we will analyze in further detail. On the contrary, a study on childhood obesity reports no significant associations between *UCP2 A55V* and BMI or body composition. The exon 4 C to T variant at amino acid 55 seems to be of a little or no importance in the regulation of child and adult body weight (18). A study of young Korean children found that subjects with the T/T genotype of *UCP2 A55V* have a lower BMI than those with the C/C and C/T genotypes. Ethnic differences in body weight and

fat distribution, and gene polymorphism frequencies may possibly explain these discrepancies.

The small number of subjects that participated in our study may not be able to give the definite conclusion. However, this was the first preliminary study about the uncoupling protein genes in relation with energy expenditure in obese Indonesian children. This study suggests that the subjects with the T/T genotypes of *UCP3-55C/T* and *UCP2 A55V* have lower TEE than those with other genotypes. Despite the shown result on the metabolic efficiency, the contribution of physical activity to TEE needs to be further studied.

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