UCP2 I/D Modulated Change in BMI During a Lifestyle Modification Intervention Study in Japanese Subjects

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Aim: Polymorphisms in uncoupling protein (UCP) genes have been strongly associated with energy expenditure and obesity. This study aimed at investigating the effects of UCP gene polymorphisms (UCP1 –3826A/G, UCP2A/V, UCP2 I/D, and UCP3 –55C/T) on change in body mass index (BMI) during a lifestyle modification program in Japanese subjects. **Results:** Intervention induced a significant decrease in energy intake ($-8.6\% \pm 17.0\%$) and a significant increase in energy expenditure ($7.7\% \pm 7.4\%$). As a result, participants experienced a significant decrease in their BMI of $-1.8\% \pm 2.7\%$. In a multivariate regression analysis, only UCP2 D/I among the selected UCP gene polymorphisms was associated with a change in BMI independent of the effects of gender, age, baseline BMI, changes in energy intake, and expenditure. Further regression analysis revealed that, in contrast to the DD genotype group, the DI+II genotype group showed no significant association between weight loss and change in energy expenditure suggesting this polymorphism altered the effects of this parameter on change in BMI. **Conclusion:** The study found UCP2 D/I to be associated with change in BMI by altering the effect of change in energy expenditure on change in BMI.

Introduction

THE RATE OF OBESITY has been increasing in Japan due to recent changes in social environment and lifestyle (Yoneda *et al.*, 2008). Weight loss, even in modest amounts, that is, 5%, has been demonstrated to be effective for managing obesity-related disorders (De Luis *et al.*, 2008). Such reduction can be achieved through lifestyle modification intervention that promotes normal weight. To increase the success of intervention, the participants' genetic background should be taken into account as it has been recognized that genetic factors may account for 60%–90% of body mass index (BMI) variance within a population (Hinney *et al.*, 2010) and some genetic variants have been demonstrated to have substantial influence on weight change (Deram and Villares, 2009).

For that purpose, the role of variants in uncoupling protein (UCP) genes could be of interest. The UCP gene family is comprised of five homologous genes (UCP1 to UCP5) that encode for mitochondrial protein carriers, which promote net translocation of protons from the inter-membrane space, across the inner mitochondrial membrane to the mitochondrial matrix. This dissipates the potential energy available for the conversion of ADP to ATP and decreases ATP production (Souza *et al.*, 2011). These genes have been shown to be involved in energy expenditure, and glucose and lipid oxidation (Dulloo and Samec, 2000; Souza *et al.*, 2011), all of which contribute to the regulation of body weight.

Several studies have reported, though discrepantly, the association of obesity with UCP single-nucleotide polymorphisms (SNPs), including UCP1 –3826A/G (rs1800592) (Nakano *et al.*, 2006; Jia *et al.*, 2010), UCP2 –55 A/V (rs660339), a 45bp insertion–deletion polymorphism in the UCP2 gene (UCP2 I/D) (rs1800795), and UCP3 –55C/T (rs1800849) (Shiinoki *et al.*, 1999; Hamada *et al.*, 2008; Kosuge *et al.*, 2008; Jia *et al.*, 2009; Matsunaga *et al.*, 2009). However, studies on the association between these SNPs and intervention-induced weight loss are few and, except for UCP1 –3826A/G (Nagai *et al.*, 2011), none of these studies was conducted on Japanese subjects. Given the differences among ethnic groups regarding the frequency and the linkage disequilibrium pattern of genetic polymorphisms, it is felt these above-mentioned findings need to be specifically replicated in the Japanese population before applying them in future intervention. This investigation focused, therefore, on the hypothesis that UCP1 –3826A/G, UCP2 A/V, UCP2 I/D, and UCP3 –55C/T modulate weight change during a lifestyle modification study in the Japanese population.

Materials and Methods

Subjects

Participants were voluntarily selected for a 3-month behavioral change interventional program for obesity prevention during the years 2000 to 2010 in Izumo City, Shimane

Prefecture, Japan. For purposes of this study, 164 subjects (38 men, age: 58.8 ± 8.2 years and 126 women, age: 56.4 ± 7.9 years) with complete data were selected. The ethics committee of Shimane University School of Medicine approved all the study protocols and all subjects gave written informed consent.

Participants underwent a program for 3 months, including recommendation of diet recipes, promotion of exercise, and support through group therapy. Data on demographic factors and lifestyles were obtained using a self-reported questionnaire. Participants were encouraged to record pedometer measurements daily (HJ-002; Omron Co. Ltd.), and their body weight weekly.

Diet

Information on the participant's daily diet was obtained using an established self-administered quantitative food frequency questionnaire before and after the 3-month behavioral intervention. Trained nutritionists asked the participants to report their weekly consumption of food, and the average amount and frequency of food intake for 1 month were estimated regarding the 30 kinds of food groups based on a report from the Working Committee for Health Guideline of the Japanese Ministry of Health and Welfare for Japanese people. An average daily nutrient intake (kcal/day) for 1 month was calculated using the standard food composition tables for Japanese (The Science and Technology Agency of Japan, 2009).

Physical activity

Habitual physical activity was assessed before and after the intervention using a questionnaire. This questionnaire evaluated physical activity, including physical exercise during leisure time as well as walking. Participants were asked to submit the pedometer measurements for the 1 week before as well as during the intervention program. Daily energy expenditure (kcal/day) was calculated using metabolic equivalent units (MET) by the following formula: calories of physical activity = body weight (kg) $\times$ metabolic equivalent (MET) $\times$ time (h). The MET for sitting/resting was 1 and that of normal walking was 3. MET values were used for a variety of physical activities, based on estimations set out in the Exercise and Physical Activity Guide from the Ministry of Health, Labor and Welfare of Japan (The Office for Lifestyle-Related Diseases Control, 2006).

Anthropometric measurements

Before and after the intervention, and following an overnight fast, the participants underwent anthropometric evaluations. The subject's body weight with very light clothing was measured to an accuracy of ± 0.2 kg, and the height was measured to an accuracy of ± 0.5 cm using a height bar. BMI was computed as weight (kg) divided by squared height (m^2).

DNA analysis

Genomic DNA was prepared from leukocytes using a DNA Extractor WB Kit (Wako Pure Chemical). *UCP1* -3826A/G (rs1800592), *UCP2* A/V (rs660339), *UCP2* I/D (rs1800795), and *UCP3* -55C/T (rs1800849) were genotyped by polymerase chain reaction-restriction fragment length polymorphism.

Genotyping platform details are showed in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/gtmb).

Statistical analysis

For each polymorphism, participants were divided into risk allele carriers and normal genotype groups referring to each polymorphism previously reported associated with BMI. The independent Student's *t*-test was used to assess differences between genotype groups at baseline. The paired Student's *t*-test was used to assess whether *UCP* polymorphisms had altered response to the intervention. Multiple linear regression analysis was done to investigate if the change in BMI was independently associated with *UCP* polymorphisms except for gender, age, changes in energy intake, and expenditure. To further assess whether the independent association of *UCP* polymorphism(s) with change in BMI was by altering the effect of change in lifestyle factors on change in BMI, multivariate regression analysis was run by genotype subgroups. A nominal two-sided *p*-value of less than 0.05 was used to assess significance. Analysis of data was done with SPSS statistical analysis software (Version 12; SPSS Japan, Inc.).

Results

The frequency of minor alleles of other *UCP* polymorphisms was 52% (AA: 38/164, AG: 83/164, and GG: 43/164), 24% (II: 9/164, ID: 60/164, and DD: 95/164), 44% (AA: 50/164, AV: 83/164, and VV: 31/164), and 29% (CC: 86/164, CT: 60/164, and TT: 18/164) for *UCP1* -3826A/G, *UCP2* D/I, *UCP2* A/V, and *UCP3* -55C/T, respectively. The genotype distribution of *UCP1* -3826A/G ($\chi^2 = 0.03$; $p > 0.05$), *UCP2* A/V ($\chi^2 = 0.01$; $p > 0.05$), *UCP2* I/D ($\chi^2 = 0.11$; $p > 0.05$), and *UCP3* -55C/T ($\chi^2 = 2.22$; $p > 0.05$) did not deviate from Hardy-Weinberg equilibrium.

UCP polymorphisms and baseline BMI

As shown in Table 1, at baseline, participants showed similar values of studied parameters between genotype groups of each gene polymorphism except for BMI values between normal and risk allele carriers subgroups of *UCP1* -3826A/G ($p = 0.018$) and *UCP3* -55C/T ($p = 0.022$). A regression analysis subsequently confirmed these associations were independent of age, gender, energy intake, and expenditure (Table 2).

UCP polymorphisms and BMI change

The intervention induced a significant decrease in energy intake ($-8.6\% \pm 17.0\%$, $p < 0.0001$) and a significant increase in energy expenditure ($7.7\% \pm 7.4\%$, $p < 0.0001$). As a result, participants experienced a significant decrease compared to their baseline BMI values of $-1.8\% \pm 2.7\%$ ($p < 0.0001$). None of the *UCP* polymorphisms altered the participants' response to the intervention (Table 1). Multivariate regression analysis (Table 3 showed that, of the selected potent independent effectors, baseline BMI ($p = 0.0003$), energy intake ($p < 0.0001$), energy expenditure ($p < 0.0001$), and *UCP2* D/I ($p = 0.006$) were independent predictors of change in BMI. To further check if *UCP2* D/I effect on change in BMI was by altering the effect of change in lifestyle factors on change in BMI, regression analysis was done separately in normal and risk allele

TABLE 1. RESPONSE TO THE BEHAVIOR CHANGE COUNSELING BY *UCP* GENE POLYMORPHISMS

	Normal			Risk allele carriers			
	Baseline	After	p (B vs. A)	Baseline	p (BN vs. BR)	After	p (B vs. A)
<i>UCP1</i> -3826 A/G		38 (AA)				126 (GG + AG)	
Age (year)	55.1 ± 7			57.8 ± 8.09			
Energy intake (kcal/day)	2008 ± 381	1781 ± 401	<0.0001	1983 ± 452	0.937	1783 ± 415	<0.0001
Energy expenditure (kcal/day)	1871 ± 514	2003 ± 559	<0.0001	1786 ± 459	0.268	1923 ± 487	<0.0001
BMI (kg/m ²)	24 ± 2.6	23.6 ± 2.5	<0.0001	25.3 ± 3.09	0.018	24.8 ± 2.94	<0.0001
<i>UCP2</i> I/D		95 (DD)				69 (DI + II)	
Age (year)	56.7 ± 7.6			57.8 ± 8.39			
Energy intake (kcal/day)	1986 ± 454	1778 ± 435	<0.0001	1994 ± 421	0.753	1799 ± 384	<0.0001
Energy expenditure (kcal/day)	1757 ± 379	1879 ± 412	<0.0001	1872 ± 578	0.127	2028 ± 608	<0.0001
BMI (kg/m ²)	24.7 ± 2.9	24.4 ± 2.8	<0.0001	25.4 ± 3.24	0.187	24.75 ± 3.03	<0.0001
<i>UCP2</i> A55V		50(AA)				114 (AV + VV)	
Age (year)	57.4 ± 7.8			57 ± 8.06			
Energy intake (kcal/day)	1955 ± 416	1745 ± 366	0.0002	2005 ± 450	0.501	1805 ± 432	<0.0001
Energy expenditure (kcal/day)	1737 ± 386	1861 ± 419	<0.0001	1836 ± 508	0.22	1977 ± 539	<0.0001
BMI (kg/m ²)	25 ± 2.8	24.7 ± 2.7	<0.0001	25 ± 3.2	0.936	24.49 ± 3	<0.0001
<i>UCP3</i> -55C/T		78 (CT + TT)				86 (CC)	
Age (year)	58.8 ± 9.2			57 ± 7.8			
Energy intake (kcal/day)	1965 ± 341	1699 ± 271	0.0004	1993 ± 451	0.381	1797 ± 427	<0.0001
Energy expenditure (kcal/day)	1896 ± 415	2021 ± 468	<0.0001	1794 ± 482	0.236	1932 ± 512	<0.0001
BMI (kg/m ²)	24.1 ± 2.9	23.6 ± 2.5	<0.0001	25.1 ± 3.05	0.022	24.66 ± 2.94	<0.0001

Data are expressed as mean ± SD. $p < 0.05$.

p (B vs. A): p -values between baseline vs. after calculated by paired Student's t -test.

p (BN vs. BR): p -values between baseline values of normal vs. risk allele carriers, calculated independent Student's t -test.

BMI, body mass index; UCP, uncoupling protein.

genotype groups of *UCP2* D/I (Table 3). The result in the normal genotype group was similar to the total population, whereas in risk allele carriers genotype, baseline BMI ($p = 0.095$) and change in energy expenditure ($p = 0.089$) did not show an independent association with change in BMI; this suggests that *UCP2* D/I affected change in BMI by altering the effect of change in expenditure on change in BMI.

Discussion

In the present study, after the 3-month lifestyle modification program, carriers of the DI + II genotype of *UCP2* D/I lowered their BMI to a lesser extent than did the DD genotype carriers. This effect resulted from altering the effect of change in energy expenditure on BMI change. Review of the literature indicates that this is the first time this association has been reported in Japanese. However, in Caucasians, one study (Papazoglou *et al.*, 2012) recently found that *UCP2* D/I was

associated with weight loss during a lifestyle modification, but contrary to the findings herein, carriers of DI + II had a more favorable response to the intervention compared to DD genotype carriers. Another previous study (Berentzen *et al.*, 2005) found no weight-loss differences between the two genotype groups. The heterogeneity among these prior studies and the present one may be attributable to differences in ethnicity and study settings, notably sample sizes and participants' inclusion criteria regarding age, gender, and obesity status.

The Insertion-Deletion polymorphism is located in exon 8 in the 3' untranslated region of the *UCP2* gene. It was reported that this mutation affects *UCP2* mRNA stability *in vitro* (Esterbauer *et al.*, 2001) and, could therefore, have a functional like effect by decreasing *UCP2* transcription. However, this functionality effect was not observed *in vivo* (Walder *et al.*, 1998). Previous studies have, although with discrepancy, found an association between weight loss and other polymorphisms in the *UCP2* gene (Sesti *et al.*, 2005; Cha *et al.*, 2007; Chen *et al.*, 2007). The possibility cannot be excluded that these polymorphisms may be in linkage disequilibrium with *UCP2* D/I, or that all these associations may be the result of a yet to be discovered functional polymorphism in the *UCP2* gene or in nearby genes (Yoon *et al.*, 2007; De Luis *et al.*, 2009).

The underlying mechanism by which *UCP2* modulates change in BMI also remains to be clarified. One possible scenario is that *UCP2* D/I is associated with resting energy expenditure; however, there was insufficient proof of influence on BMI as no association was found between this gene polymorphism and BMI (Walder *et al.*, 1998). In addition, another study also demonstrated that *UCP* genes expressions were not correlated with energy expenditure, but rather with fatty acid oxidation (Schrauwen *et al.*, 2000). This action could be

TABLE 2. MULTIVARIABLE LINEAR REGRESSION OF FACTORS INFLUENCING BODY MASS INDEX AT BASELINE

	Adj. R ²	F	β	p
	0.16	6.3		<0.0001
Gender			-0.46	0.649
Age (year)			0.38	0.706
Energy intake (kcal/day)			1.19	0.237
Energy expenditure (kcal/day)			3.98	<0.0001
<i>UCP1</i> -3826 A/G			-2.85	0.005
<i>UCP3</i> -55C/T			-2.05	0.042

Adj. R², adjusted R².

p -values of variants associated with baseline BMI were expressed.

TABLE 3. MULTIVARIABLE LINEAR REGRESSION WITH WEIGHT LOSS BY THE INTERVENTION

					UCP2 I/D (DD)				UCP2 I/D (DI+II)			
	Adj. R ²	F	β	p	Adj. R ²	F	β	p	Adj. R ²	F	β	p
	0.29	8.3		<0.0001	37.9	9		<0.0001	13	3.8		0.003
Gender			−0.03	0.688			−0.99	0.326			−0	0.97
Age (year)			−0.02	0.755			0.31	0.76			−1.2	0.249
BMI baseline (kg/m ²)			−0.21	0.003			−2.69	0.009			−1.7	0.095
Energy intake (kcal/day)			0.35	<0.0001			3.99	<0.0001			2.81	0.006
Energy expenditure (kcal/day)			−0.26	<0.0001			−3.07	0.003			−1.7	0.089
UCP1 −3826 A/G			0.04	0.598								
UCP2 I/D			0.21	0.006								
UCP2 A55V			0.13	0.071								
UCP3 −55C/T			−0.04	0.555								

p-values of variants associated with weight loss were expressed.

mediated by certain events that cause calorie depletion (Souza *et al.*, 2011). In this regard, it was reported that *UCP2* and *UCP3* expressions were increased 2- to 2.5-fold by calorie restriction in human adipose and skeletal muscle (Millet *et al.*, 1997). These findings suggest that *UCP* genes could play a role in switching of adipose and muscle metabolism. Thus, during calorie-depletion events such as, in this study, enhanced physical activity and reduced energy intake causing reduced availability of glucose, *UCP* genes could play a role in a switch to more fat utilization. This could explain the modulation of weight loss by *UCP2 D/I* during the intervention.

The association between *UCP1* −3826A/G and *UCP3* −55C/T and BMI was replicated in the current study. The association between *UCP1* −3826A/G and BMI contradicts previous studies conducted in the Japanese population (Hayakawa *et al.*, 1999; Kahara *et al.*, 2002; Kotani *et al.*, 2008; Tsunekawa *et al.*, 2011) with the exception of two studies (Matsushita *et al.*, 2003; Nakano *et al.*, 2006). It is worth noting that in the latter two studies, such association was inconsistent. In one, the relation with obesity only concerned premenopausal women (Matsushita *et al.*, 2003), but not postmenopausal women, while in the other, the difference was found only between AG heterozygous and AA wild type and not between GG mutant homozygous and AA wild type (Nakano *et al.*, 2006). Regarding the association between *UCP3* −55C/T and BMI, the present study's findings are in accord with the previous studies conducted on Japanese (Hamada *et al.*, 2008; Matsunaga *et al.*, 2009). It has also been reported that, in addition to the association with *UCP3* −55C/T, there were associations of haplotypes (H5 and H6) of this polymorphism with *UCP2 A55V* and *UCP3* rs2075577 (Kosuge *et al.*, 2008). Again, reasons for discrepancies in outcomes may be attributed to the heterogeneity in these studies settings such differences in sample size, and participants' inclusion criteria, such as age, gender, and obesity status.

This study is not exempt from measurement error of lifestyle factors, given the difficulty of measuring complex factors, such as diet and physical activity, especially when self-reported. Further, considering the relatively limited size of our sample, our outcomes may be prone to statistical errors.

In summary, the present study found that *UCP2 D/I* was associated with change in BMI and this association was by modulating change in energy expenditure effect on change in

BMI. Therefore, it is recommended that future intervention should take in to account this polymorphism for a better outcome.

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Author Contributions

K.S. designed the research; P.B.M., M.Y. and K.S. conducted the research; P.B.M. analyzed data and wrote the article; and P.B.M. had the primary responsibility for the final content. All authors read and approved the final manuscript.

Author Disclosure Statement

All authors have no conflicts of interest.

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