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Effects of a Weight Loss Program on Body Composition and Resting Energy Expenditure according to UCP 2 Genotype in Overweight Subjects*

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The objective of this study was to examine the effects of a weight loss program on the degree of obesity and levels of resting energy expenditure (REE) in overweight subjects according to their mitochondrial uncoupling protein 2 (UCP 2) genotype. Twenty-three subjects with a body mass index (BMI) greater than 27 were recruited from the Obesity Clinic of the Kyung-Hee University Hospital during the period of December 2000 - August 2001. The subjects were genotyped for the exon 8 allele; 15 subjects were found to be of *del/del* genotype, 8 were del/ins, and none were of ins/ins genotype. No significant association was found between the different UCP 2 genotypes and the initial levels of weight, fat mass (FM), lean body mess (LBM), BMI, REE, and REE/LBM ratio. After 12 weeks of a weight loss program, body weight and FM were significantly decreased, while LBM, total body water (TBW), and REE were not changed, irrespective of UCP 2 genotype. Initial fasting plasma levels of albumin, glucose, triglyceride, lipoprotein cholesterol, insulin, free triiodothyronine (T3), free fatty acid (FFA), and leptin were not different according to the UCP 2 genotype; furthermore, these blood parameters were not changed after the 12-week weight loss program. However, plasma levels of leptin decreased in both the *del/del* and *ins/del* genotypes, from 18.7 ng/ml to 13.4 ng/ml ($p<.05$), and from 18.1 ng/ml to 13.9 ng/ml ($p<.05$), respectively, after the weight loss program. In conclusion, this study found no significant association between the *del/del* or del/ins UCP 2 genotypes and differing levels of REE or differing degrees of obesity, either before or after a weight loss program. This study provided evidence that a well- managed weight loss program could maintain levels of REE, which plays an important role in the maintenance of energy balance.

Key Words : Obesity, UCP 2 genotype, polymorphism, resting energy expenditure, weight loss program

INTRODUCTION

Obesity is a common and intractable problem in some modern societies. Body weight is normally regulated by food intake and energy expenditure.[1] A low resting metabolic rate, a low rate of fat oxidation, and low levels of physical activity are all risk factors for obesity.[2)-5)] Resting energy expenditure (REE) is generally defined as the energy consumed by an individual at rest after overnight fasting. REE is approximately 10% above basal energy expenditure (BEE).[6] The BEE, the obligatory metabolic cost for the maintenance of physiological process and cellular functions, normally accounts for approximately 60% of total energy expenditure.[4),7)] Factors which affect the rate of energy expenditure might be of importance in the development of obesity.[8]

A substantial part of the REE derives from a leaking of protons across the mitochondrial inner membrane,[5),6),10)] which results in energy dissipation because of the uncoupling of oxygen consumption for ATP synthesis.[11),12)] Because obesity is presumed to develop when there is an imbalance between energy intake and energy expenditure, candidate genes for body weight regulation include those that might be important for the regulation of energy expenditure, such as those that might affect thermogenesis.[6),13)] Therefore, the uncoupling proteins (UCPs), which may translocate protons into the mitochondrial matrix, resulting in heat generation without ATP synthesis,[14] have been examined for their associations with body weight.[15),16)] The human genetic locus containing the recently-cloned UCP 2 and UCP 3 genes has been linked to several factors that are believed to be relevant for the regulation of body weight in adults.[15] REE, weight gain, and possibly the percentage of body fat, have all been found to have genetic linkage to the

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UCP 2/UCP 3 locus.[14),17)-19)]

The purpose of this study was to investigate the effects of a well-planned weight loss program on body weight, body composition, and REE according to UCP 2 genotype.

SUBJECTS AND METHODS

1. Subjects

Twenty three subjects, 6 males and 17 females, were recruited from those patients with a body mass index (BMI) greater than 27 kg/m^2 at the Obesity Clinic of the K University Hospital during the period December 2000 to August 2001. The subjects, who were already recommended by the Obesity Clinic to participate in the weight loss program, were free of medical diseases. All subjects were prescribed a low calorie diet (1200 kcal/day) for 12 weeks. Each day, the subjects were given two meals containing a total of 1000 kcal, and a further meal of Medi Food (Korea Medical Foods Co., Ltd) containing 200 kcal, to meet the recommended daily allowance for vitamins and minerals.

2. Weight Loss Program

During the 12-weeks of the weight loss program, subjects visited the Obesity Clinic once every 2 weeks. The program consisted of six 30-45 minute sessions. The subjects were instructed by a qualified dietitian to select their own foods and to modify their food behavior during the study period. The written and verbal instructions included information on the low-calorie-diet, increased physical activity, awareness of personal eating habits, and strategies for the maintenance of proper body weight. To achieve the 1,200 kcal/day energy restriction, a dietitian explained to each subject the food exchange system, which is an easy way for the subjects to monitor their food intakes. Dietary record-assisted recalls were conducted, before and after the 12 week weight loss program, to assess the dietary intakes of the subjects. Nutrient intakes were analyzed by the nutritional analysis program (CAN pro, The Korean Nutrition Society, Korea).

3. Anthropometric Measurements

Height, weight, fat mass (FM), lean body mass (LBM), and body mass index (BMI) were measured by a Body Fat Analyzer (TBF-202, Japan). These measurements were taken when subjects were dressed in light clothing and no shoes, and were recorded to the nearest 0.1 cm or 0.1 kg. The circumferences, including upper arm, forearm, waist, hip, thigh, calf, and wrist, were measured with measuring tape and recorded to the nearest 0.1 cm. Skin-fold thickness, including triceps, subscapular, suprailiac, abdomen, and thigh, were measured by a skinfold caliper and recorded to the nearest 0.1 mm; the average of two measurements was taken as the final result.

4. Resting Energy Expenditure (REE)

REE was measured by a portable indirect calorimeter (Metavine-N, Vine Bio-Dynamic Systems, Inc. Tokyo, Japan) when the subjects were awake and in a sitting position. Subjects, who had not eaten or drunk for 2 hours or more before the measurement of their REE, were wearing a face mask and breathed normally for 3 minutes. The average of three measurements was taken as the final value. The room temperature ranged from 20℃ to 25℃.

5. Biochemical analysis

Fasting blood samples were taken from the subjects before and after the weight loss program. The following biochemical parameters were measured using an Auto-analyzer (Hitachi 747, Japan): albumin, total protein, glucose, BUN, total lipid, triglyceride, total cholesterol, HDL-cholesterol, and VLDL-cholesterol. Plasma LDL-cholesterol levels were calculated by the Fridewaid fomula[20]: LDL-cholesterol = Total-cholesterol - (Triglyceride/5 + HDL-cholesterol). Plasma insulin levels were measured by radio immunoassay (RIA) with polyethylene glycol separation.[21] Fasting plasma leptin levels were determined by RIA using a commercially available kit (Linco Research. St. Louis. Mo., USA).[22] Fasting plasma FFA levels were measured by the colorimetric method.[23]

6. Genotyping the polymorphism of UCP 2

Genotyping of the UCP 2 polymorphism was undertaken with PCR using the following primers: 5' -CAGTGAGGGAAGTGGAGG-3' and 5'-GGGGCAGGACGAAGATTC-3'. The primers amplified a product of 457 bp (insertion allele) or 412 bp (deletion allele).[24] Polymerase chain reaction amplification was carried out in a volume of 24$\mu\ell$ containing 100 ng genomic DNA, 1 x PCR buffer, 0.2 μ mol/l of each primer, 0.2 mmol/LdNTP, 2.0 mmol/L$MgCl_2$ and 0.313 unit Amplitaqpolymerase (Perkin Elmer. Foster City, Calif., USA). The cycling program was an initial denaturation at 95℃ for 3 minutes, at 95℃ for 50 seconds, annealing at 60℃ for 50 seconds. and elonaturation at 72℃ for 50 seconds by 30 cycles, using a Gene-Amp 9600 Thermal Cycler (Perkin Elmer, USA). The PCR-products were resolved on a 2% agarose gel.

7. Statistical analysis

Statistical analyses were undertaken using the Statistical Analysis System (SAS) version 6.12. All values

are given as the mean±SD. The paired t-test was used for comparison of the data before and after the weight loss program. The one-way ANOVA was applied for comparison purposes during the 12 weeks of the weight loss program. The non-parametric U Mann-Whitney test for unpaired values was used for comparisons between the different groups of subjects. The $P<0.05$ level was considered statistically significant.

RESULTS

1. General characteristics of the subjects

Table 1 shows the general characteristics of the 23 obese subjects, grouped according to UCP 2 genotype; regarding the exon 8 ins/del allele, 15 subjects were found to be homozygous (*del/del*), 8 subjects were heterozygous (*ins/del*), and no subjects were found to be homozygous (*ins/ins*). The mean weights of the *del/del* and *del/ins* subjects were 78.0±8.9 kg and 78.3±8.4 kg, respectively. The mean LBM of the *del/del* and *del/ins* subjects were 47.9±4.7 kg and 47.0±7.3 kg, respectively. The mean REE of the *del/del* and *del/ins* subjects were 1873.9±364.4 kcal/day and 1960.4±225.9 kcal/day, respectively. The measures of obesity such as weight, FM, LBM, BMI, REE, and REE/LBM ratio were not significantly different between the UCP 2 genotype groups.

Table 1. General characteristics of subjects grouped by UCP 2 genotypes

	del/del (n=15)	del/ins (n=8)
Age (yrs)	40.3±9.3[1)]	43.4±10.6
Height (cm)	163.2±5.5	168.2±4.8
Weight (kg)	78.0±8.9	78.3±8.4
WHR	0.92±0.06	0.91±0.04
FM[2)] (kg)	28.9±6.1	31.5±7.5
Body fat (%)	37.3±6.3	40.1±5.6
LBM[3)] (kg)	47.9±4.7	47.0±7.3
BMI[4)] (kg/m2)	29.4±2.7	29.9±1.0
REE[5)] (kcal/day)	1873.9±364.4	1960.4±225.9
REE/LBM (kcal/day·kg(LBM)$^{-1}$)	39.0±5.3	42.1±4.1

1) Values are mean±SD
2) FM: fat mass
3) LBM: lean body mass
4) BMI: body mass index
5) REE: resting energy expenditure

2. Body weight and body composition of the subjects

Fig 1 presents the weight and body composition of the subjects during the 12-wk weight loss program, grouped by UCP 2 genotype. Following the weight loss program, subjects significantly reduced their body weight from 78.0±8.9 kg to 74.6±9.0 kg in *del/del* subjects ($p<0.05$) and from 78.3±8.4 to 73.4±6.3 kg in *del/ins* subjects ($p<.05$). Fat mass also decreased from 28.7±5.8 to 24.9±5.6 kg in *del/del* subjects ($p<.05$) and from 31.5±7.5 to 24.9±4.9 kg in *del/ins* subjects ($p<.05$). In spite of these reductions in weight and fat mass, lean body mass and total body water were not changed.

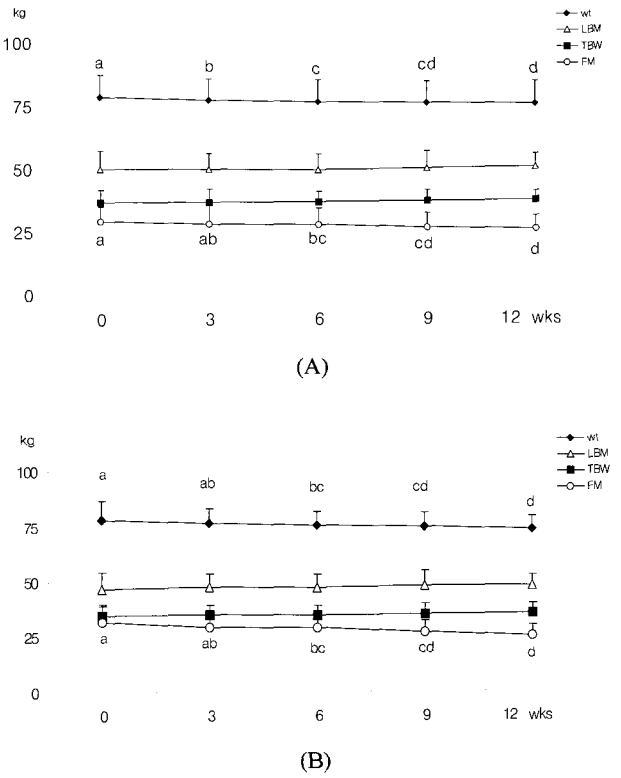
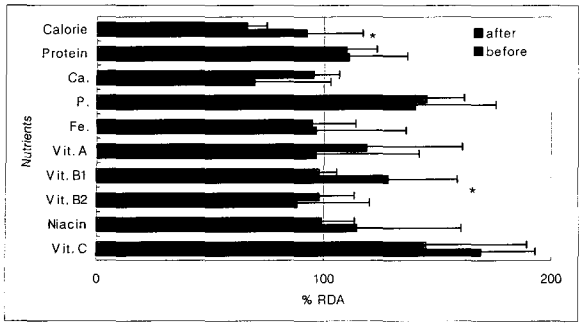


Fig 1. Body weight and body composition in *del/del* (A) and *ins/del* (B) subjects

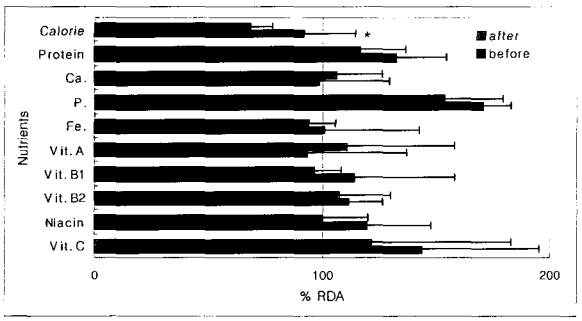
Means with the different alphabets (a, b, c, d) are significant at $p<0.05$ by Duncan's Multiple Range Test.

3. Nutrient intakes of the subjects

Fig 2 presents a comparison of subjects' nutrient intakes with the RDA for nutrients before and after the weight loss program. The total calorie intakes of the *del/del* subjects and *ins/del* subjects significantly decreased from 1875.6±449.3 kcal (92% of RDA) to 1272.5±122.5 kcal (66% of RDA : $p<0.05$) and from 1939.1±521.4 kcal (92% of RDA) to 1299.5±80.0 kcal (68.2% of RDA : $p<0.05$), respectively. In the *del/del* subjects, vitamin B_1 intakes decreased from 1.3 mg (128% of RDA) to 0.9 mg (96% of RDA : $p<0.05$). While the total calorie intakes of both groups were below the RDA, most of the nutrient intakes of both groups were similar to the RDA levels, after the 12 week weight loss program.



(A)



(B)

Fig 2. Comparison of nutrient intakes with RDA in *del/del* (A) and *ins/del* (B) subjects before and after the 12 week weight loss program

*p<0.05

RDA (Recommanded dietary allowances) for Korean, 7th revision, 2000

4. Anthropometric parameters of the subjects

Table 2 shows results for the anthropometric parameters of the subjects grouped by UCP 2 genotype before and after 12 weeks of the weight loss program. Anthropometric measurements were not significantly different according to the UCP 2 genotype. The measurements of upper arm, forearm, waist, hip, and thigh were significantly decreased after the 12-week program in *del/del* subjects. The measurements of waist and thigh were significantly decreased in *del/ins* subjects ($p<0.05$). In skin-fold thickness, all parameters including triceps, subscapular, suprailiac, abdomen, and thigh were significantly decreased after the 12 week program in *del/del* subjects ($p<0.05$). Subscapular and suprailiac measurements were significantly decreased after 12 weeks in *del/ins* subjects ($p<0.05$); however, triceps, abdomen and thigh measurements did not change in *del/ins* subjects.

5. Resting energy expenditure (REE) and REE/LBM ratio of the subjects

Table 3 summarizes results for the REE and the REE/LBM ratio, grouped by UCP 2 genotype before and after the weight loss program. These measurements were not significantly different according to UCP 2 genotype and were not changed in both groups after 12 weeks of the weight loss program.

Table 2. Anthropometric measurements of subjects grouped by UCP 2 genotypes before and after the 12 week weight loss program

	del/del (n=15)		ins/del (n=8)	
	Before	**After**	**Before**	**After**
Circumference (cm)				
Upperarm	32.1±1.5	30.1±2.1*	32.4±1.2	30.7±2.5
Forearm	25.2±1.4	24.6±1.4*	25.5±1.9	24.7±1.0
Waist	96.2±9.8	92.3±8.3**	95.9±5.3	91.7±5.7*
Hip	104.5±5.1	101.0±4.4**	105.8±4.7	103.5±2.7
Thigh	62.1±3.2	60.0±3.1*	62.3±2.6	60.0±3.2*
Calf	39.7±1.6	39.3±1.5	39.3±2.5	38.6±1.9
Wrist	16.8±0.6	16.6±0.8	17.1±0.8	16.7±0.5
Skinfold thickness (mm)				
Triceps	30.3±6.0	26.8±7.4*	32.5±8.5	31.1±7.6
Subscapular	39.0±6.8	32.6±8.6*	43.8±4.4	37.0±4.7*
Suprailiac	37.0±5.3	28.4±9.7*	42.3±8.7	34.5±9.1*
Abdomen	39.5±6.2	34.3±8.3*	43.2±4.1	39.0±10.0
Thigh	34.7±7.4	28.9±6.5*	39.2±5.1	32.4±7.9*

1) Values are mean ± SD
2) * $p < 0.05$
** $P < 0.001$

Table 3. REE and REE/LBM values of subjects grouped by UCP 2 genotypes before and after the 12 week weight loss program

	del/del (n=15)		ins/del (n=8)	
	Before	**After**	**Before**	**After**
REE (kcal/day)	1937±374.6	1930.6±370.3	1929.9±224.8	1914.7±313.9
REE/LBM (kcal/day · kg(LBM)$^{-1}$)	39.7±6.8	39.1±7.0	41.7±4.3	40.1±5.8

REE : Resting energy expenditure
LBM : Lean body mass

Table 4. Blood parameters of subjects grouped by UCP 2 genotypes before and after the 12 week weight loss program

	del/del (n=15)		del/ins (n=8)	
	Before	**After**	**Before**	**After**
Total Protein (g/dl)	7.4±0.4[1)]	7.4±0.4	7.4±0.3	7.5±0.2
Albumin (g/dl)	4.6±0.2	4.6±0.3	4.7±0.1	4.7±0.3
Glucose (mg/dl)	90.2±12.1	90.5±11.8	83.7±8.3	85.7±6.1
BUN (mg/dl)	12.3±2.6	12.5±4.4	15.7±4.3	17.6±4.0
Total lipid (mg/dl)	652.3±93.7	610.8±156.2	649.7±120.9	629.7±78.9
Triglyceride (mg/dl)	169.3±105.4	166.5±120.5	128.4±67.0	123.3±65.5
Total cholesterol (mg/dl)	181.5±15.7	175.0±25.4	204.7±46.5	196.0±21.1
HDL-cholesterol (mg/dl)	39.1±6.6	38.9±5.5	42.7±12.6	47.3±10.7
LDL-cholesterol (mg/dl)	108.6±22.9	102.8±31.0	136.3±40.9	124.1±19.4
VLDL-cholesterol (mg/dl)	33.9±21.1	33.3±24.1	25.7±13.4	24.7±13.1
Insulin (μ Iu/ml)	13.0±6.9	13.4±5.3	8.3±7.2	8.7±6.2
leptin (ng/ml)	18.7±6.6	13.4±4.2*	18.1±6.6	13.9±4.9*
free-T3 (pg/ml)	0.6±0.5	0.9±0.3	0.5±0.2	0.9±0.6
FFA (μ Eq/L)	723.9±253.6	663.7±167.2	759.9±257.3	555.0±154.4

1) Values are mean±SD
2) * $p < 0.05$

6. Blood parameters of the subjects

Table 4 summarizes the results for the blood parameters of the subjects. Initial fasting plasma concentrations of albumin, glucose, triglyceride, and lipoprotein cholesterol were all in the normal range and were not significantly different according to UCP 2 genotype. The levels of these parameters were not modified significantly by the 12 weeks weight loss program. Initial fasting plasma insulin, free triiodothyronine, and FFA levels were not significantly different according to UCP 2 genotypes; these were also not changed by the 12 weeks of the weight loss program. However, plasma leptin concentration decreased from 18.7 ng/ml to 13.4 ng/ml ($p<0.05$) in *del/del* subjects, and from 18.1 ng/ml to 13.9 ng/ml ($p<0.05$) in *ins/del* subjects after the 12 week program.

DISCUSSION

It is a well known fact that weight loss is generally accompanied by a decrease in REE.[25)] LBM is the most important contributor to REE. Consequently, REE is frequently expressed relative to LBM. Body weight reduction obtained by caloric restriction is associated with a decrease in REE.[26-29)] This adaptive mechanism may account for the poor long-term efficacy of weight reduction programs.[30)] However, this study found that decreases in body weight by a well-planned weight loss program did not reduce REE; LBM did not decrease in response to weight loss by the 12-week weight loss program.

The subjects of this study were free of significant medical diseases, and their fasting plasma concentrations of albumin, glucose, triglyceride, and lipoprotein cholesterol were not modified by the weight loss program. Furthermore, the blood parameter levels measured in this study were not significantly different between the *del/del* and *ins/del* genotypes. Caloric restriction could induce an increase in adipose tissue lipolysis,[22,31)] resulting in release of fatty acids from the body fat stores. Fasting or very low calorie diets (VLCD) have been associated with increases in plasma FFA levels.[31)] However, in this study, plasma FFA levels did not change with weight reduction. It could be speculated that the extent of the

reduction in calorie intake achieved in this study might not be sufficient to increase the plasma FFA level.

The fact that weight loss triggers a decrease in plasma leptin is now well recognized.[22),32)] In this study, there was a significant decrease in leptin levels in response to weight loss, in both *del/del* and *ins/del* genotypes.

In other studies,[10),18),33),35)] in which the UCP 2 polymorphisms were examined in adults, there were different results with regard to the importance of these polymorphisms for body weight and energy expenditure. In a study of adult French Canadians, Otabe et al[33)] found no significant difference in the frequency of the *ins/del* gene between morbidly obese and non-obese subjects; also, no relationship was found between the UCP 2 *ins/del* genotype and body composition or REE. Walder et al[24)] found that subjects carrying the exon 8 *ins/del* heterozygote had higher metabolic rates than either *del/del* or *ins/ins* homozygotes, and that the *ins/del* genotype had been associated with a lower BMI in subjects aged over 45 years. In a study of Caucasian children,[34)] allele frequencies of exon 8 or exon 4 polymorphisms were not significantly different between children characterized by low REE and children with high REE. On the other hand, Yanovski et al[35)] found that body composition and anthropometric measurements of children were significantly different according to the UCP 2 genotypes, *ins/del* and *del/del*.[35)]

In this study, polymorphisms of the UCP 2 genotype were not associated either with level of REE or degree of obesity. There are several potential explanations for discrepancies found in this and in similar studies. Firstly, the subjects' differences in age, race, and experimental diets might underlie these disparate results. Secondly, UCP 2 polymorphisms might not be in linkage disequilibrium with another nearby important genetic variant, such as polymorphisms in the UCP 3 gene found in other studies to be associated with UCP 2, REE, and obesity.[18),35)] Bouchard et al[18)] found that the UCP 2 polymorphic marker D11S611 was tightly linked to the resting metabolic rate in a Quebec family study. Finally, regions important for gene regulation might not have been included in the present study. Thus, alleles responsible for the linkage found in other studies[18),35)] could be present in the promoter region or other regulatory parts of the UCP 2 gene or in the very closely located UCP 3 gene. Thus, the UCP 2 gene may still be important for the variability of metabolic rate and obesity.

In summary, in spite of significant weight loss, a well-planned weight loss program could maintain LBM and REE, which play such an important role in the maintenance of energy balance. Polymorphisms of the UCP 2 gene might not affect REE and might not contribute to obesity. These results, however, do not exclude the possibility that variants in regulatory elements of the gene could contribute to the development of obesity.

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