Low-Calorie Diet-Induced Reduction in Serum HDL Cholesterol Is Ameliorated in Obese Women with the -3826 G Allele in the Uncoupling Protein-1 Gene

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The uncoupling protein-1 (UCP1) gene is of major importance for regulation of body weight and lipid/lipoprotein metabolism. Our cross-sectional study has shown that subjects with the G/G genotype of the -3826 A/G polymorphism in the UCP-1 gene have higher levels of serum high-density lipoprotein cholesterol (HDL-C) than those with other genotypes. Low circulating HDL-C level has been regarded as a major atherosclerotic risk factor. We therefore investigated whether the -3826 A/G polymorphism affects the obesity- and lipid-related parameters during a low-calorie diet (LCD) intervention. In 32 obese women (49.9 ± 8.4 years of age), anthropometric, physiological and biochemical characteristics were measured before and after a 2-month LCD treatment, which restricted each subject to the same energy intakes, such as 5,120 kJ/day. The -3826 A/G polymorphism was detected using a PCR-restriction fragment-length polymorphism method. There were 6 subjects with the A/A genotype, 15 with the A/G genotype and 11 with the G/G genotype. The LCD intervention decreased weight ($P < 0.001$) and serum HDL-C levels ($P < 0.05$) in all subjects. There was no difference in the levels of change in weight, nutrient intake, physiological measurements in energy expenditure, and fat oxidation between subjects with and without the G allele. In contrast, the degree of the reduction in the HDL-C levels was significantly smaller in subjects with the G allele than those without the G allele. These results suggest that the G allele at -3826 in the UCP1 gene may ameliorate the reduction in serum HDL-C levels in obese women during LCD. ——— uncoupling protein-1; weight reduction; lipid metabolism; nutrition; polymorphism.

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A recent worldwide problem is the increasing number of people with obesity and related metabolic disorders such as diabetes mellitus and dyslipidemia, leading to atherosclerotic disease (Kopelman 2000). Obesity and lipid disorders are caused by imbalances in energy intake and expenditure (Spiegelman and Flier 2001; Lovegrove and Gitan 2008). Energy homeostasis is maintained by signals from feedback loops that regulate food intake, energy expenditure, and lipid and energy metabolism (Spiegelman and Flier. 2001). Currently, it remains to be clarified which genetic variants among many genes contribute to such disorders, and to

what degree (Kopelman 2000; Del Mar Gonzalez-Barroso et al. 2000)

A low-calorie diet (LCD) is the preferred tool for treating obesity (Finer 2001; Astrup et al. 2004). Meal replacements have been used with palatable liquid meals substituting as a self-selected, self-assembled food source as well as providing reproducible energy and macronutrient composition (Finer 2001; Astrup et al. 2004). The LCD-based approach aims to reduce weight through a decrease in fat mass while preserving lean body mass and to improve obesity-related metabolic risk factors. There may be individual

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differences based on genetic factors in the effects of a LCD on obesity and related metabolic disorders.

Concerning genetic factors, uncoupling protein-1 (UCP1) and its gene are generally assumed to play an important role in body weight regulation and in the pathogenesis of obesity and metabolic disorders (Del Mar Gonzalez-Barroso et al. 2000; Argyropoulos and Harper 2002). The role of UCP1-mediated thermogenesis in human obesity remains unclear (Argyropoulos and Harper 2002), but the presence of the -3826A/G polymorphism in the promoter region of the UCP1 gene has been associated with increased weight gain (Oppert et al. 1994), lower weight loss under a low-calorie diet (Fumeron et al. 1996), and other lipid-related metabolic features of the obese phenotype (Proenza et al. 2000). In addition, the relationship between the -3826A/G polymorphism within the promoter region of the UCP-1 gene and lipid/lipoprotein metabolism has been studied (Esterbauer et al. 1998; Kieć-Wilk et al. 2002; Matsushita et al. 2003; Oh et al. 2004; Kotani et al. 2008).

Interestingly, the UCP1 genotype may be associated with the metabolism of lipids such as high-density lipoprotein cholesterol (HDL-C), a major contributor to atherosclerotic disease (Oh et al. 2004). However, results concerning the relationship between the -3826 A/G polymorphism and HDL-C have not been consistent (Esterbauer et al. 1998; Kieć-Wilk et al. 2002; Matsushita et al. 2003; Oh et al. 2004; Kotani et al. 2008). It has been reported that the G allele or the GG genotype of the UCP-1 gene is associated with lower HDL-C levels compared to the A/A or the A/G + A/A genotype (Oh et al. 2004; Kotani et al. 2008). Our previous study has shown the G/G genotype to be an independent protective factor associated with low HDL-cholesterol in Japanese individuals (Kotani et al. 2008). These studies were carried out using a cross-sectional design, which may explain the discrepancies. To date, the genetic effects of the -3826A/G polymorphism on metabolic changes caused by a LCD have not been investigated with an interventional design.

Therefore, an interventional study was designed to examine the genetic effects of the -3826A/G polymorphism in the UCP gene on obesity- and lipid-related metabolic changes during a LCD intervention in obese women. We hypothesized that the -3826A/G polymorphism might contribute to changes in several obesity- and lipid-related parameters.

Subjects and Methods

Subjects

Thirty-two obese women (mean $\pm$ s.d.; age 49.9 ± 8.45 years; BMI 28.4 ± 3.3 kg/m²) participated in this study, approved by the Ethics Committee of the Kyoto Medical Center. Obesity was determined as a BMI ≥ 25 kg/m² according to criteria for Japanese (The Examination Committee of Criteria for Obesity Disease in Japan, Japan Society for the Study of Obesity, 2002). The subjects completed a health questionnaire regarding medical history, medications, and

lifestyle. The subjects were not clinically diagnosed with hypertension, dyslipidemia, diabetes mellitus, cardiovascular disease or other endocrine diseases, nor were they taking medications that could influence weight change, autonomic function, or glucose/lipid metabolism. This study did not include subjects who were pregnant or breast feeding, abused alcohol or drugs, or had psychological contraindications. No subject was taking nutritional supplements or hypersensitive to any ingredients of the formula, including but not limited to soy protein.

LCD intervention

The LCD intervention was designed to achieve a 5% reduction in initial body mass over an 8-week period. During the study period, the subjects were restricted to 5021 kJ/d, consisting of two regular meals for breakfast and lunch, and one meal replacement with a formula diet (diet's, Suntory. Ltd., Osaka, Japan) for dinner. The subjects chose from five flavors (strawberry, cocoa, green tea, banana, and mixed fruit). These meals provided an average of 178.0 ± 0.5 kcal, 21.1 ± 0.2 g protein, 1.3 ± 0.04 g fat, 17.7 ± 0.4 g carbohydrate, 5.5 ± 0.2 g dietary fiber, and 208.8 ± 3.0 mg sodium. The amounts of macro- and micro-nutrients including 12 vitamins and 9 minerals met more than a third of dietary standards for Japanese. Trained dietitians counseled the individuals in planning daily balanced meals. All subjects visited our institution to confirm adherence to the diet, and food diaries were reviewed, along with weight charts, which the subjects were required to record at home. Each subject received sufficient meal-replacement formula to last until the next scheduled visit. To scrutinize the nutritional intake in the LCD intervention, mean energy and other intakes were calculated from 3-day food records using Micronutrient software (Healthy Maker Pro501, Mushroomsoft Co. Ltd., Okyama, Japan) at the time of biochemical measurements.

Physiological experiment

Data for all subjects were examined before and after the intervention. The subjects were asked not to consume any food or beverages containing alcohol or caffeine after 9:00 p.m. on the day preceding the examination, and also instructed to abstain from alcohol use and excessive physical activity for 24 to 48 hours before the examination. On the day of the examination, the subjects came to the laboratory at approximately 9:00 a.m., and all experiments were performed in the morning. The room was kept at 25°C, quiet, and comfortable, with a minimization of arousal stimuli. Body mass and body fat were measured using a body fat analyzer (OMRON, Co. Ltd., Kyoto, Japan). Seated blood pressure levels were measured three times at 10-minute intervals using a mercury sphygmomanometer (HEM-5001, OMRON, Co. Ltd., Kyoto, Japan). At least 20 minutes prior to starting the experiment, respiratory exchange measures were determined using an indirect open-circuit indirect calorimeter (Aero monitor AE-300SRC, Minato Medical Science, Tokyo, Japan). The calorimeter was calibrated before each test with a reference gas mixture (15% O₂ and 5% CO₂). The mean for a stable 8-minute measurement period was calculated for resting energy expenditure (EE). The respiratory quotient (RQ) was determined from the $\dot{V}O_2$ and the $\dot{V}CO_2$ output of the calorimeter. EE was calculated using the table of Lusk (1924).

Biochemical analysis

Blood samples drawn from the antecubital vein were immediately transferred to siliconized tubes containing disodium EDTA (1 mg/ml), and centrifuged at 4°C. Plasma glucose levels were mea-

sured by the hexokinase method (SHINO-TEST Co. Ltd., Tokyo, Japan), and serum insulin concentrations were measured by chemiluminescent immunoassay (Bayer-medical. Co. Ltd., Tokyo, Japan). Serum total cholesterol levels (Wako Pure Chemical Industries, Co. Ltd., Tokyo, Japan), HDL-C, low-density lipoprotein (LDL)-cholesterol, and triglyceride levels (DAIICHI PURE CHEMICALS Co. Ltd., Tokyo, Japan), and nonesterified free fatty acid (NEFA) levels (Wako Pure Chemical Industries, Co. Ltd., Tokyo, Japan) were determined by enzymatic methods.

Genetic analysis of the -3826A/G polymorphism in the UCP1 gene

A noninvasive genotyping method has been implemented for carefully collecting buccal mucosa cells without contamination using cytobrushes (Nagai et al. 2003). After the phenol-extraction procedure, 0.2 to 2 μ g of DNA was obtained per subject. The -3826A/G polymorphic region of UCP1 (rs1800592) was amplified using the polymerase chain reaction (PCR) with a sense primer (5'-TTT GTG CAG CGA TTT CTG ATT GAC -3'), an anti-sense primer (5'-AGG AGT GGC AGC AAG TTC TGA ATG -3'), and a primer labeled at the 5' end with Texas red (5'-AAA TGC ACT CGA TCA AAC TGT-3'). PCR was performed under the conditions recommended by the enzymes supplier. In brief, a 25- μ l aliquot contained 20 ng of genomic DNA, the reaction buffer supplied with the enzyme, 3.0 mM MgCl₂, 0.2 mM dNTP, and 1.25U of rTaq containing anti-Taq high (TOYOBO Co. LTD., Thuruga, Japan). The cycling parameters were as follows: an initial denaturation at 95°C for 5 min, then 40 cycles of the denaturation at 95°C for 30 sec, annealing at 65°C for 30 sec, and primer extension at 72°C for 30 sec, followed by post extension at 72°C for 2 min in a thermal cycler (ABI9700). A 2- μ l reaction mix-

ture containing 10 pmol of probe and SYBR Green I (final concentration $\times 10,000$) was added to the PCR products. The mixture was put into the ABI PRIZM 7700 and the melting temperature was measured. The program for analytical melting was 95°C for 30 sec, 40°C for 1min, and an increase to 80°C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 nm and 612 nm, respectively.

Statistical analyses

All data are expressed as the mean $\pm$ s.d. Hardy-Weinberg equilibrium based on allele frequency was tested with the chi-square test. The t-test was performed to test differences between the two groups. A two-way ANOVA (before and after the intervention by genotype) was used to identify the interventional changes to parameters by genotype. *P* values less than 0.05 were accepted as statistically significant. All statistical analyses were performed using the Statistical Package of Social Science (SPSS for windows, version 11.0, SPSS Inc., Chicago, Illinois).

Results

The frequency of the G allele at the -3826A/G polymorphism was 58% and in Hardy-Weinberg equilibrium. The distribution of the A/A, A/G, and G/G genotypes was 18%, 49%, and 33%, respectively. All subjects completed the intervention. Table 1 illustrates the obesity- and lipid-related metabolic changes during the intervention. Weight and body fat decreased significantly. Both systolic and diastolic blood pressure dropped significantly as well. In addi-

Table 1. Changes in obesity-and lipid-related parameters after a low-calorie diet intervention in all obese subjects.

	Before	After	delta	<i>P</i> value
Body weight (kg)	69.6 $\pm$ 8.0	64.9 $\pm$ 7.46	-4.65 $\pm$ 1.98	0.001
Body height (cm)	157 $\pm$ 5.38	157 $\pm$ 5.38	0.00 $\pm$ 0.00	
Body fat (%)	36.5 $\pm$ 2.68	34.4 $\pm$ 3.38	-2.03 $\pm$ 1.50	0.003
Body mass index (kg/m ²)	28.4 $\pm$ 3.26	26.5 $\pm$ 3.23	-1.86 $\pm$ 0.75	0.011
Systolic blood pressure (mmHg)	129 $\pm$ 17.1	125 $\pm$ 16.1	-4.19 $\pm$ 10.8	0.035
Diastolic blood pressure (mmHg)	79.8 $\pm$ 11.5	74.8 $\pm$ 10.8	-4.94 $\pm$ 6.94	0.007
HbA1c (%)	4.93 $\pm$ 0.32	4.96 $\pm$ 0.30	0.03 $\pm$ 0.19	0.454
Fasting blood glucose (mmol/L)	5.04 $\pm$ 0.62	4.80 $\pm$ 0.51	-0.24 $\pm$ 0.35	0.001
Total cholesterol(mmol/L)	5.67 $\pm$ 0.93	5.27 $\pm$ 0.99	-0.40 $\pm$ 0.36	0.001
LDL-cholesterol (mmol/L)	3.17 $\pm$ 0.74	2.90 $\pm$ 0.74	-0.27 $\pm$ 0.31	0.005
HDL-cholesterol (mmol/L)	1.67 $\pm$ 0.39	1.58 $\pm$ 0.30	-0.09 $\pm$ 0.22	0.018
Triglyceride(mmol/L)	2.47 $\pm$ 1.68	2.16 $\pm$ 1.48	-0.31 $\pm$ 0.77	0.029
Non-esterified fatty acids (mEq/l)	0.62 $\pm$ 0.20	0.79 $\pm$ 0.29	0.17 $\pm$ 0.37	0.015
Insulin (pmol/L)	11.6 $\pm$ 10.9	7.36 $\pm$ 4.89	-4.23 $\pm$ 7.61	0.004
Oxygen consumption (l/min)	0.21 $\pm$ 0.03	0.18 $\pm$ 0.03	-0.03 $\pm$ 0.03	0.011
Oxygen consumption (ml/kg/min)	3.06 $\pm$ 0.43	2.82 $\pm$ 0.36	-0.23 $\pm$ 0.39	0.002
Respiratory quotient	0.83 $\pm$ 0.03	0.78 $\pm$ 0.02	-0.05 $\pm$ 0.03	0.005
Energy expenditure (kcal/min)	1.01 $\pm$ 0.13	0.87 $\pm$ 0.13	-0.14 $\pm$ 0.13	0.001
Fat oxidation (mg/min)	64.2 $\pm$ 17.2	69.7 $\pm$ 13.5	5.42 $\pm$ 12.0	0.015
Carbohydrate oxidation (mg/min)	98.5 $\pm$ 28.0	51.1 $\pm$ 17.1	-47.3 $\pm$ 26.3	0.012
Total oxidation (mg/min)	163 $\pm$ 21.6	121 $\pm$ 19.7	-41.9 $\pm$ 24.8	0.011

Values are means $\pm$ s.d. *P* values indicate the comparison between before and after diet intervention.

Table 2. Changes in obesity- and lipid-related parameters after a low-calorie diet intervention in obese female subjects according to UCP1 genotype.

	Subjects without the G allele (<i>n</i> = 6)				Subjects with the G allele (<i>n</i> = 26)				* <i>P</i> value A/A vs. A/G, G/G
	Before	After	delta	<i>P</i> value	Before	After	delta	<i>P</i> value	
Body weight (kg)	69.9 ± 11.7	65.2 ± 10.7	-4.75 ± 2.12	0.001	69.5 ± 7.19	64.9 ± 6.80	-4.63 ± 2.00	0.001	0.894
Body fat (%)	36.1 ± 3.4	33.3 ± 4.27	-2.80 ± 1.47	0.005	36.5 ± 2.56	34.7 ± 3.18	-1.85 ± 1.48	0.002	0.164
Body mass index (kg/m ²)	28.9 ± 5.1	27.0 ± 4.65	-1.95 ± 0.85	0.002	28.3 ± 2.82	26.4 ± 2.92	-1.84 ± 0.75	0.002	0.750
Systolic blood pressure (mmHg)	127.0 ± 18.4	122.2 ± 15.2	-4.83 ± 9.54	0.70	129.2 ± 17.1	125.2 ± 16.6	-4.04 ± 11.2	0.257	0.874
Diastolic blood pressure (mmHg)	78.3 ± 15.9	72.0 ± 13.3	-6.33 ± 4.50	0.14	80.1 ± 10.6	75.5 ± 10.4	-4.62 ± 7.42	0.057	0.593
HbA1c (%)	5.10 ± 0.40	5.07 ± 0.34	-0.03 ± 0.35	0.97	4.90 ± 0.30	4.93 ± 0.30	0.04 ± 0.13	0.724	0.641
Fasting blood glucose (mmol/L)	4.90 ± 0.54	4.65 ± 0.42	-0.17 ± 0.48	0.10	5.10 ± 0.64	4.80 ± 0.53	-1.06 ± 0.32	0.057	0.425
Total cholesterol (mmol/L)	5.42 ± 1.17	4.85 ± 1.28	-0.57 ± 0.28	0.002	5.72 ± 0.92	5.37 ± 0.92	-0.35 ± 0.37	0.001	0.173
LDL-cholesterol (mmol/L)	2.89 ± 0.89	2.53 ± 0.76	-0.04 ± 0.34	0.035	3.23 ± 0.70	2.98 ± 0.72	-0.25 ± 0.30	0.002	0.428
HDL-cholesterol (mmol/L)	1.89 ± 0.64	1.63 ± 0.49	-0.26 ± 0.35	0.025	1.62 ± 0.30	1.56 ± 0.25	-0.06 ± 0.16	0.463	0.041
Triglyceride (mmol/L)	1.87 ± 0.49	1.83 ± 0.87	-0.04 ± 0.83	0.998	2.61 ± 1.83	2.23 ± 1.59	-0.38 ± 0.77	0.090	0.376
Non-esterified fatty acids (mEq/l)	0.63 ± 0.19	0.73 ± 0.15	0.10 ± 0.23	0.905	0.61 ± 0.21	0.79 ± 0.32	0.18 ± 0.40	0.078	0.637
Insulin (pmol/L)	11.3 ± 13.2	5.48 ± 3.61	-5.77 ± 9.72	0.277	11.7 ± 10.6	7.80 ± 5.10	-3.88 ± 7.23	0.069	0.592
Oxygen consumption (l/min)	0.21 ± 0.03	0.18 ± 0.03	-0.03 ± 0.02	0.035	0.21 ± 0.03	0.18 ± 0.03	-0.03 ± 0.03	0.002	0.729
Oxygen consumption (ml/kg/min)	3.07 ± 0.29	2.81 ± 0.30	-0.26 ± 0.25	0.411	3.05 ± 0.46	2.82 ± 0.38	-0.23 ± 0.42	0.057	0.882
Respiratory quotient	0.83 ± 0.03	0.79 ± 0.02	-0.04 ± 0.01	0.022	0.83 ± 0.04	0.78 ± 0.02	-0.05 ± 0.03	0.001	0.104
Energy expenditure (kcal/min)	1.02 ± 0.16	0.87 ± 0.13	-0.15 ± 0.09	0.029	1.01 ± 0.12	0.87 ± 0.14	-0.14 ± 0.13	0.001	0.817

Values are means ± s.d. *P* values indicate the comparison between before and after a low-calorie diet intervention. **P* values indicate the comparison of delta change between the genotypes. There was a significant difference in delta change in HDL-C levels between the two genotypes (*P* = 0.041).

tion, there were significant reductions in total cholesterol, LDL-cholesterol, triglyceride, insulin, and fasting blood glucose levels.

Within each group, weight, body fat, body mass index, systolic and diastolic blood pressure, fasting glucose concentration, total- and LDL-cholesterol levels, and whole body carbohydrate oxidation were significantly reduced following the intervention. There was no difference in the changes of any physiological and metabolic parameters between the subjects with and without the A allele, and between the subjects with the A/G genotype and those with the G/G genotype (data not shown). However, in the analyses between two groups (Table 2), changes in HDL-C levels differed significantly by genotype. Namely, while changes in nutritional intake and obesity-related parameters such as weight, body fat, and body mass index, did not differ between the groups, the degree of the reduction in the HDL-C levels was significantly smaller in the subjects with the G allele than those without the G allele. Physiological changes in energy expenditure, and carbohydrate and fat oxidation did not differ between subjects with and without the G allele (data not shown).

From the recorded data, dietary protein, fat, and carbohydrate intake levels were significantly reduced in both groups. However, the degree of the reduction was not significantly different between subjects with and without the G allele: subjects without the G allele (protein -14.9 ± 7.6 g, fat -35.3 ± 26.2 g, and carbohydrate -88.7 ± 26.9 g) and subjects with the G allele (protein -9.3 ± 15.9 g, fat -23.7 ± 25.2 g, and carbohydrate -80.8 ± 51.9 g). With respect to changes in physical activity, alcohol intake, smoking, breakfast, snacks between meals and beverages containing caffeine, there was no difference between subjects with and without the G allele (data not shown).

Discussion

Low circulating HDL-C levels are regarded as a major atherosclerotic risk factor (Gordon et al. 1977; Gordon et al. 1989; Kitamura et al. 1994) via the interplay of environmental, nutritional, and genetic factors (Williams et al. 1993, 1995; Watts 2001). In the present study, circulating HDL-C levels were reduced to a significantly less extent by a LCD in subjects with the G allele than those without the G allele, although weight loss and nutrient intake levels did not differ between genotypes. The results of this study suggest a predictive association between the G allele at -3826 in the UCP1 gene and the metabolism of HDL-C during a LCD intervention. A LCD intervention often results in a reduction in circulating HDL-C levels. This has been discussed with respect to the incidence of cardiac events (Katzel et al. 1995). The clinical implications of our finding that the reduction in serum HDL-C levels was less extensive in subjects with the G allele than those without the G allele remain undetermined. Moreover, the influence of genotype should be considered and the -3826A/G polymorphism is a predictor for the regulation of HDL-C levels during calorie restric-

tion.

Results of previous cross-sectional studies of the relationships between the -3826A/G polymorphism and lipid disorders have been controversial (Oh et al. 2004; Kotani et al. 2008). The present study adopted an interventional design; therefore, the results better support the involvement of the -3826A/G polymorphism in the metabolism of HDL-C. Previous research has indicated that, whereas changes in the oxidation of free fatty acids in mitochondria may alter circulating lipid levels through tissue cholesterol transport, energy homeostasis in mitochondria may be affected by genetic polymorphisms (Oh et al. 2004). During calorie restriction, increased whole-body oxidation of dietary fatty acids/lipids is regulated by L-carnitine, which carries long-chain fatty acyl groups into mitochondria for β -oxidation (Demizieux 2005). The present results indicated no association between the UCP1 genotype and changes in physiological parameters, which may be related to the consumption of oxygen and oxidation of fat/carbohydrates in mitochondria during the LCD intervention. In this study, the respiratory quotient of the intra-group comparison before and after intervention was significantly reduced, while that of the inter-group comparison tended to have a greater reduction in subjects with the G allele than in those without the G allele. The present results may indicate a difference in the utilization of lipids as a source of energy between subjects with the A allele and those with the G allele. Further study is needed to clarify the connection between the -3826A/G polymorphism and the metabolism of lipids including HDL-C.

In conclusion, the present findings suggest that the G allele at -3826 in the UCP1 gene may ameliorate the reduction in serum HDL-C levels in obese women during LCD. Since the present study was limited by a small number of samples, studies with a larger number of samples and more sophisticated designs should be conducted.

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