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Genetic polymorphisms of the renin-angiotensin system and obesity-related metabolic changes in response to low-energy diets in obese women

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ABSTRACT

Objective: Genetic polymorphisms of the renin-angiotensin system have been implicated in cardiovascular and metabolic diseases. The purpose of this study was to investigate whether the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene and 3123C/A polymorphism of the angiotensin II type 2 receptor (AT_2R) gene affect blood pressure and other obesity-related metabolic changes in response to low-energy diets using meal replacement shakes for weight loss.

Methods: Clinical, metabolic, and biochemical profiles were measured before and after a 2-mo intervention in 32 obese women (age 49.9 ± 8.4 [SD] y; BMI 28.4 ± 3.3 kg/m^2) restricted to 1200 kcal/d (5021 kJ/d). The polymorphisms were determined with an intercalater-mediated FRET probe assay system.

Results: Although weight loss and nutrient intake levels did not differ among the genotypes, the reduction in body fat after weight loss was significantly less in the ACE deletion/deletion (D/D) genotype than insertion/insertion (I/I) plus I/D genotype (−2.25 ± 1.40% versus −0.80 ± 1.57%, $P < 0.05$). The AT_2R A/A group had significantly less improved levels of systolic blood pressure (−7.23 ± 8.50 versus 2.50 ± 12.6 mmHg, $P < 0.05$), low-density lipoprotein-cholesterol (−0.36 ± 0.29 versus −0.09 ± 0.25 mmol/L, $P < 0.05$), carbohydrate (−54.4 ± 27.2 versus −31.8 ± 16.3 mg/min, $P < 0.05$) and fat oxidation (8.31 ± 11.86 versus 0.05 ± 9.99 mg/min, $P < 0.05$) than the C/C plus C/A genotypes.

Conclusion: The present findings suggest that the homozygous form of the ACE gene may hinder the improvement of body fat and that the homozygous form of the AT_2R gene may make improving systolic blood pressure and some obesity-related metabolic parameters through a dietary intervention difficult among obese women.

Introduction

The world epidemic of obesity has resulted primarily from an imbalance of caloric intake and expenditure in societies becoming more affluent and sedentary [1,2]. A current major concern addresses metabolic syndrome, a cluster of abnormalities including excess abdominal adiposity, dyslipidemia, high blood pressure, and disturbances in glucose homeostasis [3,4]. Even as the Japanese enjoy Methuselah-like life expectancies, the 2006 Annual Report of the National Health and Nutrition Survey highlighted some disturbing trends: approximately 19 million Japanese between 40 and 72 y of age showed some signs of metabolic syndrome [5]. Successful strategies for reducing obesity, especially fat deposition or blood pressure, together with improving obesity-related metabolic risk factors are of utmost importance. However, for any given individual, human obesity is a multifactorial trait influenced by environmental,

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nutritional, and genetic factors [1]. In making strategies, the inclusion of genetic factors is presently thought to be crucial.

Dietary calorie restriction is a basic treatment for obesity that aims to induce weight loss through low-energy diets and so avoid the risks and poor results associated with starvation [6,7]. However, there are considerable individual differences in the effects of dieting on obesity-related metabolic profiles, the causes of which have not been fully established.

Concerning genetic factors, polymorphisms in several candidate obesity-related genes have been the target of intensive investigation, and some polymorphisms of the renin-angiotensin system (RAS) genes have been implicated in cardiovascular and metabolic diseases [8–10]. The strong link between hypertension and obesity has also led to interest in the RAS. Angiotensin-converting enzyme (ACE) plays a major role in the regulation of blood pressure and is recognized as a key component of the RAS [11]. A widely prevalent ACE insertion/deletion (ACE I/D) gene polymorphism characterized by the presence or absence of a 287-base-pair (bp) fragment in the human ACE gene accounts for half of the variance in circulating ACE levels [12]. Thus, the ACE gene may occupy a central place in the RAS system with a role in relation to blood pressure, and in turn, cardiovascular and metabolic diseases. Furthermore, up to now, most human studies have been on angiotensin II type 1 receptor (AT_1R) genotypes and related effects. The angiotensin II type 2 receptor (AT_2R) is also reportedly involved in hypertension or other obesity-linked metabolic parameters [8]. To date, associations of the 3123 Cytosine/Adenine (3123C/A) polymorphism (which is in the 3′ untranslated region of exon 3 of the X-chromosome-located AT_2R gene) with essential hypertension, myocardial infarction, and hypertrophic cardiomyopathy have been reported [13–17]. However, the genetic effects of RAS polymorphisms on improvements of blood pressure and the metabolic profile in response to weight loss after a low-energy diet intervention have not been clarified in obese people.

Therefore, the purpose of the present study was to investigate whether insertion/deletion polymorphism of the ACE gene and 3123C/A of the AT_2R gene affect blood pressure and other metabolic changes related to obesity through a dietary intervention by low-energy diets using meal replacement shakes for weight loss in obese women. It was hypothesized that these polymorphisms of the RAS genes may contribute to the individual variation in some metabolic changes after a dietary intervention.

Subjects and methods

Subjects

Thirty-two asymptomatic obese Japanese women (mean ± SD; age 50 ± 9 y; BMI 28.4 ± 3.3 kg/m^2) participated in this study. The study was approved by the Ethics Committee of the Kyoto Medical Center and all subjects gave their written informed consent. Obesity was defined as a BMI ≥25 kg/m^2 according to criteria for Japanese [18]. Prior to any experiments, the subjects had medical examinations and interviews and completed a health questionnaire regarding medical history, medications, current health, and lifestyle. The subjects were all nondiabetic and had normo-echocardiograms. They were not clinically diagnosed with hypertension, hyperlipidemia, cardiovascular disease, or other endocrine diseases nor were they taking medications that could influence weight loss, autonomic function, or glucose/lipid metabolism. None of the subjects was pregnant or breast feeding, abused alcohol or drugs, or suffered from psychological contraindications.

Low-energy diets with meal replacement

The participant-oriented, energy-restricted diet program was designed to produce a 5% reduction of initial body mass over a 2-mo period. During the study period, subjects were restricted to 1200 kcal/d (5021 kJ/d), consisting of two regular meals for breakfast and lunch, and one meal replacement with a formula diet (diet's, Suntory Co. Ltd., Osaka, Japan) for dinner. The subjects chose from among five flavors: strawberry, cocoa, green tea, banana, and mixed fruit. The formula provided an average value 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 nine minerals met more than a third of the Dietary Reference Intakes for Japanese (Health, Labour, and Welfare Ministry in Japan, 2005). Trained dietitians individually counseled the subjects in planning daily, nutritionally balanced meals. Subjects were seen on a fortnightly basis in the Kyoto Medical Center for dietary adherence. During these visits, body mass was measured; any difficulties adhering to this intervention were discussed, and food diaries were reviewed, along with weight charts, which all subjects were required to record at home. Each subject received sufficient meal-replacement formula to last until the next scheduled visit, plus one additional week's worth should she need to reschedule an appointment with the dieticians. To scrutinize the dietary nutritional intake throughout the low-energy diet intervention, mean (SD) energy intake and other nutritional intakes were calculated for every 4 wk from 3-d food records using Micronutrient software (Healthy Maker Pro501; Mushroomsoft Co. Ltd., Okyama, Japan). Subjects were met in individual interviews and counseled by a nutritionist.

Experimental procedure

All subjects were examined on two separate occasions: before and after the 8-wk dietary intervention. Body mass and height were measured using a body fat analyzer (OMRON, Co., Ltd., Kyoto, Japan). At the end of a gentle expiration, waist circumference was measured at the level midway between the lowest rib margin and the iliac crest. Blood pressure was measured three times at 10-min intervals using a mercury sphygmomanometer (HEM-5001; OMRON, Co. Ltd., Kyoto, Japan).

Concerning eating habits, no subject was taking nutrient supplements or hypersensitive to any ingredients of the formula, including but not limited to soy protein. The consumption of balanced meals, a daily breakfast, snacks between meals, and beverages containing caffeine were also checked. Daily physical activity was averaged as the total number of steps during the last 7 days each of the beginning, middle, and end of the low-energy diet program. The number of daily steps was calculated with a pedometer (HJ-106; OMRON, Co. Ltd.) placed on the subject's belt. In regard to the precision of pedometer measurements, the error level is established within 5%.

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 study. The subjects were also instructed to abstain from alcohol use and excessive exercise for 24 to 48 h before testing. On the day of the testing, 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. After accurate skin preparation, the subjects were fitted with electrocardiogram electrodes and rested for at least 20 min prior to the start of the experiment. Respiratory exchange was measured with an open-circuit computerized indirect calorimeter (Aero monitor AE-300SRC; Minato Medical Science, Tokyo, Japan). The calorimeter was calibrated before each test with a reference gas mixture (15% O_2 and 5% CO_2). The mean for a stable 8-min measurement period was calculated for resting energy expenditure. The respiratory quotient was determined from the oxygen consumption (VO_2) and the carbon dioxide (VCO_2) output of the calorimeter. Energy expenditure was calculated using the table of Lusk [19].

Biochemical analysis

Blood samples drawn from the antecubital vein were immediately transferred to siliconized tubes containing Na_2 EDTA (1 mg/mL) and centrifuged at 4°C. Plasma and serum were immediately frozen and stored at −20°C until the assay. The plasma glucose concentration was measured by the hexokinase method (Shino-Test Co., Tokyo, Japan), and the serum insulin concentration was assayed by chemiluminescent immunoassay (Bayermedical Co. Ltd., Tokyo, Japan). Serum total cholesterol levels (Wako Pure Chemical Industries, Co. Ltd., Tokyo, Japan), high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and triglyceride levels (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan), and concentrations of non-esterified free fatty acids (NEFA-HRII; Wako Pure Chemical Industries, Co. Ltd., Tokyo, Japan) were determined by enzymatic methods.

Determination of genetic polymorphisms

A non-invasive genotyping method has been implemented for carefully collecting buccal mucosa cells using cytobrushes without contamination [20]. After the phenol-extraction procedure, 0.2 to 2 μg of DNA per subject was