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The association between carbon and nitrogen stable isotope ratios of human hair and metabolic syndrome

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ABSTRACT

Background: It has been reported that stable isotope ratios can be used as biomarkers for animal protein intake. Meat consumption and high protein intake could be risk factors for metabolic disorders. We investigated whether the stable isotope ratios of carbon and nitrogen are associated with metabolic syndrome.
Methods: We conducted a cross-sectional study of 399 subjects (233 men and 166 women). Hair samples from 399 subjects were measured for stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$).
Results: The $\delta^{15}N$ values progressively increased with the number of components of the metabolic syndrome present in study subjects (*P* for trend 0.047). In multivariable models, $\delta^{15}N$ values were positively associated with the presence of metabolic syndrome (odds ratio, 1.53; 95% confidence interval, 1.09–2.14), whereas $\delta^{13}C$ values were not (odds ratio, 0.97; 95% confidence interval, 0.72–1.30). The odds ratio (95% confidence interval) for metabolic syndrome comparing the highest to the lowest quartiles of $\delta^{15}N$ values was 2.64 (1.17–5.92).
Conclusions: The nitrogen, but not carbon, stable isotopic ratio of hair is independently associated with the presence of metabolic syndrome. The hair $\delta^{15}N$ value might be a surrogate marker for clustering of risk factors in metabolic syndrome.

1. Introduction

Metabolic syndrome is characterized by a clustering of risk factors such as abdominal obesity, reduced high-density lipoprotein (HDL) cholesterol, elevated triglycerides (TGs), elevated blood pressure, and elevated fasting glucose. Individuals with metabolic syndrome are at an increased risk for type 2 diabetes mellitus and cardiovascular disease [1,2].

Epidemiological studies have suggested that dietary factors, including meat consumption and high protein intake, are associated with increased risks for metabolic syndrome and diabetes [3–5]. However, dietary assessments by recall methods, widely used in epidemiologic studies, have limitations in validity and precision [6]. Nutritional biomarkers for objective dietary assessment could be helpful to overcome the limitations of dietary recall methods.

Stable isotopic analyses in biological systems have provided quantitative information on food uptake into the body and the cycle of material in archeology and ecology [7]. Recently, it has been reported that stable isotope ratios can be used as nutritional biomarkers of dietary intake [8,9]. The stable isotope ratios of carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) have been shown to be associated with animal protein and meat intake [7,10–12]. However, there are few epidemiologic studies of stable isotope ratios in human populations.

Biomarkers could be useful to identify and predict the risk for chronic diseases such as metabolic syndrome, diabetes mellitus, and cardiovascular disease [13–15]. Given the high prevalence of metabolic syndrome and its potential consequences, little is known about the ability of $\delta^{13}C$ and $\delta^{15}N$ to serve as biomarkers of metabolic syndrome. We investigated whether the stable isotope ratios of carbon and nitrogen are associated with metabolic syndrome.

2. Materials and methods

2.1. Study subjects

We conducted an ancillary study of cross-sectional design within the Korean Genome and Epidemiology Study on Atherosclerosis Risk of Rural Areas in the Korean General Population (KoGES–ARIRANG), an ongoing, community-based cohort study [13]. KoGES–ARIRANG includes 5178 men and women, aged 40 to 70 y at baseline (2005–2008), of whom 3862 (74.6%) attended the first follow-up survey (April 2008–January 2011). The second follow-up survey has been in progress since 2011. We collected hair samples for the stable isotope analysis of carbon and nitrogen in 2011. The study population of the

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current analysis consisted of 547 subjects who visited the survey center from May 24, 2011 to October 6, 2011 and underwent a comprehensive health examination. We excluded 32 subjects whose diet during the past one year has been changed over the previous years, 63 subjects who were not willing to provide hair samples, and 53 subjects who did not complete informed consent for the ancillary study. Finally, 399 subjects (233 men and 166 women) were included in the data analyses. The study protocol was approved by the Institutional Review Board of Wonju College of Medicine, Yonsei University.

2.2. Measurement of anthropometric and metabolic characteristics

Comprehensive questionnaires were used to collect information, including medical and family history, health behavior, dietary intake, and physical examinations, which were performed according to standard procedures [13,16]. Body weight and height were measured while participants were wearing light indoor clothing without shoes. Waist circumference was measured in a horizontal plane, midway between the inferior margin of the ribs and the superior border of the iliac crest using a tape measure (SECA-200, SECA). Blood pressure was measured in the right arm after the participant had rested for at least 5 min in a quiet room using a standard mercury sphygmomanometer (Baumanometer). With participants seated, an appropriate-sized cuff was applied snugly around the upper right arm at heart level. The appropriate cuff size was chosen for each subject according to mid-arm circumference. Two measurements were made with at least 5-min intervals in between, and the mean of the 2 measurements was used in the analyses.

Smoking status was determined based on self-report. Non-smokers were defined as those who had smoked <100 cigarettes or 5 packs of cigarettes in their lifetime. Current smokers were defined as those who had smoked >100 cigarettes in their lifetime and also those who reported "currently smoking" in the questionnaire. Former smokers were defined as those who had smoked >100 cigarettes in their lifetime but who answered "abstain from smoking" in the questionnaire. Subjects who answered "yes" to the question: 'Do you perform physical exercise regularly enough to make you sweat?' were assigned to the 'regular exercise' group. Dietary intake was assessed by a 106-item semi-quantitative food frequency questionnaire (FFQ) developed for Korean adults [17]. The frequency of dietary intake was calculated as servings per week and average daily nutrient intake was estimated using nutrient database from the seventh edition of the Korean Food Composition Table [18]. The glycemic load was calculated by multiplying the carbohydrate content of each food item by its glycemic index and then by its frequency of intake. The total glycemic load was calculated as the sum of all food items. Each unit of dietary glycemic load represents the equivalent of 1 g of carbohydrate from glucose. The overall dietary glycemic index for a participant was calculated by dividing the daily glycemic load by the daily carbohydrate intake. The glycemic index values for individual food items were obtained from the international table [19], from the online database of the University of Sydney [20] and from published values for Korean foods [21]. The values of glycemic index and glycemic load were energy adjusted using the regression residual method to control for total energy intake.

Venous blood samples were drawn from participants in the morning after fasting overnight for 12 h. Fasting blood glucose was determined by a glucose oxidase-based assay. Serum concentrations of HDL cholesterol and TGs were determined by enzymatic methods (ADVIA 1800, Siemens Healthcare Diagnostics).

The American Heart Association, the National Heart, Lung and Blood Institute (AHA/NHLBI) and the International Diabetes Federation (IDF) definitions were adopted for the diagnosis of metabolic syndrome [1], which meant that subjects met at least 3 of the following criteria: 1) Abdominal obesity, defined as a waist circumference ≥90 cm for men or ≥85 cm for women (following Korean-specific cutoffs for abdominal obesity defined by the Korean Society for the Study of Obesity) [22]; 2) hypertriglyceridemia, defined as a serum TG concentration ≥150 mg/dl (1.69 mmol/l); 3) low HDL cholesterol, defined as a serum HDL cholesterol concentration <40 mg/dl (1.04 mmol/l) for men or <50 mg/dl (1.29 mmol/l) for women; 4) high blood pressure, defined as a systolic blood pressure ≥130 mm Hg or a diastolic blood pressure ≥85 mm Hg, or antihypertensive drug treatment; and 5) high fasting glucose, defined as a fasting serum glucose ≥100 mg/dl or drug treatment of elevated glucose.

2.3. Stable isotopic analysis

Hair samples were collected when the subjects visited the survey center in 2011. Hairs were cut from the crown of the subject's head and as close as possible to the scalp. The hair samples were prepared following a standard procedure [7,23]. Hairs were washed twice by soaking in a 2:1 mixture of methanol and chloroform for 30 min to remove sebum lipids and shampoo residue and rinsed in distilled water for 15 min. Hair samples were wrapped in aluminum foil and cut into 15-mm sections then dried overnight under a vacuum to remove moisture.

Carbon and nitrogen isotopes were measured using an isotope-ratio mass spectrometer (GV IsoPrime) interfaced with an elemental analyzer (EuroVector EuroEA3000 series) at the Korea Basic Science Institute. The elemental analyzer was based on the Dumas principle, in which dynamic flash combustion is followed by packed gas chromatography column separation of the gaseous species produced (e.g. N_2, CO_2 and SO_2) [24]. Isotopic ratios are expressed in delta (δ) notation in parts per thousand (‰) relative to the accepted international standards: Vienna Pee Dee Belemnite (PDB) for carbon isotopes and atmospheric air for nitrogen isotopes. The ratio is expressed as $\delta\,(‰) = [(R_x / R_s) - 1] \times 1000$, where R_x is the $^{13}C/^{12}C$, or $^{15}N/^{14}N$ isotopic ratio of the sample, and R_s is the $^{13}C/^{12}C$, or $^{15}N/^{14}N$ isotopic ratio of the standard. The analytical precision was ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$, respectively.

2.4. Statistical analysis

Data were checked for distributions and assessed for normality. The *t*-test, the Mann–Whitney *U* test, or the χ^2 test were conducted to compare differences in profiles of study subjects with and without metabolic syndrome, and data were expressed as means ± SD, medians (interquartile ranges), or frequencies. Spearman's rank correlation coefficients were calculated to test the correlation between $\delta^{13}C$ or $\delta^{15}N$ values and other variables. Multivariable logistic regression was used to assess the independent association of $\delta^{13}C$ or $\delta^{15}N$ values with the prevalence of metabolic syndrome. We used four models with progressive degrees of adjustment. In model 1, we adjusted for age (continuous variable) and gender (2 categories). In model 2, we additionally adjusted for smoking (3 categories) and regular exercise (2 categories). In model 3, we additionally adjusted for meat intake (servings/week), fish intake (servings/week), and total energy intake (kcal/day). In model 4, we additionally adjusted for soft drink intake (servings/week), coffee intake (servings/week), sugar added to coffee (spoons/week), tea intake (servings/week), and glycemic load. All analyses were performed using SAS version 9.3 (SAS Institute). Results were expressed as odds ratios (ORs) with 95% CI. Statistical significance was determined at a $P < 0.05$ for all comparisons.

3. Results

3.1. Anthropometric and metabolic characteristics

The prevalence of metabolic syndrome was 26.3% in the study subjects. The $\delta^{13}C$ and $\delta^{15}N$ values were significantly higher in men than in women ($\delta^{13}C = -20.07‰$ in men and $\delta^{13}C = -20.83‰$ in women, $P < 0.001$, $\delta^{15}N = 11.98‰$ in men and $\delta^{15}N = 11.03‰$ in women, $P < 0.001$). Table 1 shows the profiles of the study population.