activity. However, expression studies need to be performed to confirm this. Taking into account the allelic frequencies of the genetic polymorphisms in CYP3A4 (10% heterozygous for CYP3A4*1B, 5.4% heterozygous for CYP3A4*2, and 2.2% heterozygous for CYP3A4*3), this implies that ~15% of the (Caucasian) population may carry a genetic polymorphism in this allele. Because genetic polymorphisms may exhibit strong differences in occurrence among different ethnic groups, other populations need to be investigated to determine the allelic frequency of CYP3A4*3.

In conclusion, we have described and validated a PCR-RFLP assay for the CYP3A4*3 allele. The frequency of this variant allele in the Caucasian population (1.1%) indicates that it might be important in predicting CYP3A4 activity based on genotype. Future research should be directed toward elucidating the effect of this polymorphism on CYP3A4 enzymatic activity and toward establishing whether this is solely a genetic or also a functional polymorphism.

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References


Increases in Nitric Oxide Concentrations Correlate Strongly with Body Fat in Obese Humans, Jong Weon Choi,1,2 Soo Hwan Pai,1 Soon Ki Kim,3 Masafumi Ito,3 Chang Shin Park,4 and Young Nam Cha5 (Departments of 1 Clinical Pathology and 2 Pediatrics, College of Medicine, Inha University Hospital, Inchon, 400-103, South Korea; 3 Department of Pathology, Nagoya University Hospital, Nagoya, 466-0065 Japan; 4 Department of Pharmacology and Toxicology, Medicinal Toxicology Center, College of Medicine, Inha University, Inchon, 400-103, South Korea; * address correspondence to this author at: Department of Clinical Pathology, Inha University Hospital, 7-206, 3-ga, Shinheung-dong, Jung-gu, Inchon, 400-103, South Korea; fax 82-32-890-2529, e-mail jwchoi@inha.ac.kr)

NO is produced in many different cells and is involved in the regulation of such physiological and pathophysiological processes as inflammation, vasodilation, and metabolism (1). Depending on the cell type, NO is produced in an enzymatic reaction catalyzed by one of the three isoforms of NO synthase (NOS): neuronal NOS, endothelial NOS, and inducible NOS (2). Measurement of the NO metabolites, nitrate and nitrite (N0x), is also important as a marker of NOS enzyme activity.

Obesity is a condition involving an excess accumulation of body fat, and the prevalence of obesity is rapidly increasing worldwide. Excessive weight and obesity are leading to nutrition-related disorders of clinical and public health concern. Recent studies have suggested a role for NO in the regulation of food intake in an animal experiment (3, 4). Endothelial and inducible NOS have been shown to be present in adipose tissue of the rat (5), suggesting that adipose tissue may be a potential source of NO production. Previous reports demonstrated that NOS activity and inducible NOS protein were also present in human subcutaneous adipose tissue (6) and showed that inhibition of NOS led to increased lipolysis in this tissue (7). However, how NO production changes as body mass index (BMI) increases in apparently healthy subjects has not been studied extensively. Moreover, correlation studies of serum N0x concentrations, body fat mass, and blood lipid concentrations in healthy subjects are limited. Therefore, in the present study, we investigated the changes in N0x concentrations according to BMI and evaluated the relationships among N0x concentrations, total body fat, and lipid profiles in adolescents.
We measured serum NOx concentrations, complete blood cell counts, iron markers, and serum lipid profiles in 185 males and 178 females, 14–19 years of age, selected from 1473 students attending middle or high school. Eight anthropometric measurements were taken in all subjects: weight; height; circumference of upper chest, upper arm, waist, and hip; and subscapular and triceps skinfold thickness measured by a caliper. Body adiposity was assessed using a leg-to-leg bioelectric impedance device, the TANITA body fat analyzer (TBF-611; Tanita), which enables simultaneous measurements of body weight, impedance, fat-free mass, total body water, and fat percent-age in a subject standing on the stainless steel electrode (8). BMI was determined as weight in kilograms divided by the square of the height in meters (kg/m²). The reference interval of BMI is defined as 19.0–24.9 kg/m², overweight as a BMI of 25–29.9 kg/m², and obesity as a BMI ≥30 kg/m² (9). The males and females were divided into three groups according to BMI: overweight group (BMI ≥25.0 kg/m²), healthy weight group (19.0 ≤ BMI < 25.0 kg/m²), and underweight group (BMI <19.0 kg/m²). We compared the data in extremely lean adolescents (BMI <16.0 kg/m²) with those in obese adolescents (BMI ≥30.0 kg/m²). We excluded 24 subjects in this study—12 adolescents who showed evidence of chronic or recent infections, 7 who had histories of iron or vitamin supplementation, and 5 who had previously undergone surgical operations—because they had inflammation-induced increases in NOX. This study was approved by the Ethical Committee at Inha University Hospital. The subjects were given a detailed description of the study before their consent was obtained.

Venous blood (7 mL) was drawn into iron-free evacuated serum separator tubes after 12 h of fasting. NOX concentrations were measured by a NADPH-dependent nitrate reductase assay (10) in the serum of adolescents subjected to a reduced nitrate and nitrite diet. After the serum nitrate (NO₃⁻) was converted to nitrite (NO₂⁻) by NADPH-dependent nitrate reductase (incubated with glucose-6-phosphate dehydrogenase and NADPH in 14 mmol/L sodium phosphate buffer, pH 7.4), the total concentration of nitrite was determined spectrophotometrically at 540 nm. We measured NOX directly in the sera of adolescents, without deproteinization and without dilution because according to our results for 35 specimens selected randomly from the subjects, there were no significant differences in NOX concentrations between deproteinized and nondeproteinized sera or between diluted and nondiluted sera. To avoid diet effect on serum NOX concentrations, the subjects were given a list of foods potentially rich in nitrate and were requested to abstain from these foods before sample collection after an overnight fast. Specifically, cured meat, fish, cheese, herbal or black teas, beer, wine, and malted beverages were excluded from the diet (11).

Complete blood cell counts, serum iron markers and lipid profiles, and serum ferritin were assayed with a SE 9000 electronic counter (Sysmex), a Hitachi 747 automatic chemical analyzer (Hitachi), and an ACS:180 chemiluminescence assay (Chiron), respectively. Data analysis was conducted using the SAS 6.12 software package (SAS Institute). To compare the differences of values, we used the Kruskal–Wallis test before Mann–Whitney comparisons were made between groups. Correlation coefficients were calculated by the Spearman method. P <0.01 was considered statistically significant.

The changes in serum NOX concentrations, iron markers, and lipid profiles according to BMI are available as a data supplement at Clinical Chemistry Online (http://www.clinchem.org/content/vol47/issue6). The NOX concentrations in the underweight subjects did not differ significantly from those in subjects in the healthy weight group. However, the serum NOX concentrations were 4.1- and 4.2-fold higher in overweight male and female subjects (BMI ≥25.0 kg/m²), respectively, than in the underweight subjects (BMI <19.0 kg/m²). On the other hand, serum NOX concentrations changed in parallel with the anthropometric variables. In this study, there were no significant differences in body weight, body fat, and skinfold thickness between underweight and healthy weight subjects; however, overweight subjects showed large differences in anthropometric variables when compared with underweight adolescents. In particular, mean body fat was 1.5-fold higher in girls than in boys, whereas mean NOX concentrations were 1.3-fold higher in girls than in boys. Moreover, the NOX concentrations were 13.9-fold higher in the obese subjects (BMI ≥30.0 kg/m²) than for the extremely lean subjects (BMI ≤16.0 kg/m²).

These results indicate that NO production increases in obese human and that this increase begins from the time that BMI is >25 kg/m² in both males and females.

Because iron deficiency may up-regulate NOS activity (12), in this study we measured iron markers to strictly compare NOX concentrations between extremely lean and obese subjects. Markedly increased NOX concentrations in obese subjects (BMI ≥30.0 kg/m²), who showed no significant differences in iron markers from the extremely lean adolescents, suggest that NO biosynthesis increases overtly in obese humans irrespective of iron status. Moreover, as shown in Fig. 1, NOX concentrations correlated positively with the obesity (r² = 0.207; P <0.01) and BMI (r² = 0.211; P <0.01), and with the body fat (r² = 0.259; P <0.01) in the overweight females (BMI ≥25.0 kg/m²). Our results are in accordance with a previous report showing that NOS activity is present in human adipose tissue and produces NO through inducible NOS from a source of NO, adipose tissue per se (7). On the other hand, results contradictory to ours have also been reported. Ferlito and Gallina (13) demonstrated that in diabetics, being overweight, increased blood pressure, and diabetes mellitus per se cause a nonsignificant increase of NO production in comparison with healthy controls. Andersson et al. (14) found that plasma nitrate concentrations were not different between obese and control women. However, Ferlito and Gallina (13) measured plasma nitrite in patients with type 1 and 2 diabetes, and Andersson et al. (14) measured plasma nitrate in obese and nonobese postmenopausal women. In our study, we measured NOX in apparently
healthy adolescents 14–19 years of age without any diseases. We therefore believe that the discrepancies of the results seem to be derived from the differences in subjects among the studies.

To investigate how BMI and body fat change with serum NOx concentrations, we compared data according to NOx concentrations. There were no significant differences in anthropometric variables, iron markers, and lipid profiles between the subjects with NOx < 20 μmol/L and with NOx ≥20 μmol/L. However, the mean values of BMI, weight, and body fat showed significant differences when we compared the subjects with NOx < 20 μmol/L to NOx concentrations. There were no significant differences in anthropometric variables, iron markers, and lipid profiles between the subjects with NOx < 20 μmol/L and with NOx ≥20 μmol/L. However, the mean values of BMI, weight, and body fat showed significant differences when we compared the subjects with NOx < 20 μmol/L to

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**Fig. 1.** Scatter plots showing the correlation between serum NOx concentrations and obesity (A), BMI (B), and body fat mass (C). NOx concentrations correlated significantly with obesity (y = 1.3037x + 26.496; r² = 0.2079; P < 0.01), BMI (y = 4.2072x – 54.708; r² = 0.2118; P < 0.01), and body fat mass (y = 2.4067x – 32.808; r² = 0.2593; P < 0.01) in the 70 obese females with BMI ≥25 kg/m².
the subjects with NOx $\geq 80$ μmol/L. In particular, body fat and skinfold thickness were 1.6-fold higher in the subjects with NOx $\geq 80$ μmol/L than in the subjects with NOx <20 μmol/L. These results indicate that moderately increased NOx concentrations are relevant to BMI and body fat. In this study, we investigated whether serum lipid profiles show significant changes as NOx concentrations increase; however, unlike body fat mass, there were no significant differences in serum lipid profiles between the subjects with NOx <20 μmol/L and with NOx $\geq 80$ μmol/L.

In conclusion, our results suggest that obesity leads to increased NO production in humans. Increased serum NOx correlate strongly with body fat but poorly with serum lipid concentrations.

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In conclusion, our results suggest that obesity leads to increased NO production in humans. Increased serum NOx correlate strongly with body fat but poorly with serum lipid concentrations.

Effects of Anticoagulants and Contemporary Blood Collection Containers on Aluminum, Copper, and Zinc Results, Elizabeth L. Frank,1 Martin Patrick Hughes,2 Daniel D. Bankson,3 and William L. Roberts1 (1 Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84132; 2 Department of Laboratory Medicine, University of Washington, Seattle, WA 98195; 3 Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108; * address correspondence to this author at: c/o ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108; fax 801-584-5207, e-mail william.roberts@arup-lab.com)

Laboratory analysis is used to evaluate both deficiency and excess of trace elements (1). The intravascular concentrations of many of these elements are maintained within narrow limits. For this reason, preanalytical loss of analyte and spuriously high values attributable to contamination are concerns. Blood collection, processing, and storage before analysis are critical for accurate trace element analysis. Decreased concentrations of analytes may result from adsorption onto collection container surfaces or from the use of anticoagulants that complex metals (2, 3). Sources of contamination include patient clothing and skin; blood collection materials, including needles, anticoagulants, stoppers, serum separators, and glass containers; and particulate matter in laboratory air.

Aluminum, copper, and zinc are three metallic elements commonly monitored by the clinical laboratory. Although aluminum may have a physiological role in the action of a few enzymes, such as succinic dehydrogenase and porphobilinogen synthase, it is typically monitored to evaluate toxicity in patients subjected to hemodialysis for renal failure (3, 4). These patients may be exposed to high aluminum concentrations in their treatment regimen but lack an efficient physiological means to remove this element. Aluminum accumulation may lead to dialysis encephalopathy and osteomalacia. Toxicity is known to occur at concentrations $>100$ μg/L, although symptoms may occur at 60 μg/L or lower in dialysis patients (3, 5). Because aluminum is ubiquitous and pervasive, contamination is a serious concern for the analytical laboratory. Falsely high concentrations measured in contaminated specimens may affect clinical decisions.

Copper is an essential trace element necessary for the function of several enzymes involved in electron transport, free radical defense, and other biological oxidation-reduction reactions (1). Copper has a role in iron metabolism and is an important indicator of Wilson disease and Menkes kinky hair syndrome. Copper concentrations vary with age, gender, and ethnic group.

Zinc is an essential trace metal that is second in abundance to iron only to iron (1). Zinc is a necessary component of the active sites of many enzymes and contributes to the structural stability of numerous metalloenzymes and other proteins. More than 75% of whole blood zinc is present in erythrocytes. Hemolysis can falsely increase zinc concentration and should be avoided in specimens used to measure zinc. Copper and zinc