Nicotinuric Acid

A potential marker of metabolic syndrome through a metabolomics-based approach

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OBJECTIVE—Metabolic syndrome is a multiplex disorder and puts patients on the road to type 2 diabetes and atherosclerotic cardiovascular diseases. However, a surrogate biomarker in plasma or urine in fully reflecting features of metabolic syndrome has not been explored.

RESEARCH DESIGN AND METHODS—Urine metabolomics has potential utility in metabolic profiling because urine metabolites analysis reflects global outflux of metabolic change. Accordingly, we collected data on subjects (n = 99) with overweight, dyslipidemia, hypertension or impaired glucose tolerance and took a metabolomics approach to analyze the metabolites of urine revealed in metabolic syndrome by high-performance liquid chromatography–time-of-flight mass spectrometry and elicit potential biomarkers to picture metabolic syndrome.

RESULTS—Our results revealed that the urine nicotinuric acid value of subjects with diabetes (HbA_{1c} ≥6.5% or those receiving diabetes medications) (n = 25) was higher than subjects without diabetes (n = 37) (221 ± 31 vs. 152 ± 13 × 10^3 mAU, P = 0.0268). Moreover, urinary nicotinuric acid level was positively correlated with body mass index, blood pressure, total cholesterol, low-density lipoprotein cholesterol, triacylglycerol and high sensitivity C-reactive protein, but negatively correlated with high-density lipoprotein cholesterol.

CONCLUSIONS—This is the first study, to our knowledge, to propose that nicotinuric acid represents an important pathogenic mechanism in process from metabolic syndrome to diabetes and atherosclerotic cardiovascular disease.

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Metabolic syndrome is a multifaceted disorder and considered to be a major cause of type 2 diabetes and atherosclerotic cardiovascular diseases. Although urine metabolomics has potential utility in metabolic profiling because urine metabolites reveal a global outflux of metabolic change, a surrogate biomarker in urine that fully reflects features of metabolic syndrome has not been explored. The aim of this study was to isolate a potential biomarker of metabolic syndrome through analysis of urine metabolites in subjects with features of metabolic syndrome.

RESEARCH DESIGN AND METHODS

—Urine samples were obtained after overnight fasting from 99 unselected subjects (66 women, mean age 69 ± 13 years) who visited the hospital for a health checkup. Chromatographic separation of urine supernatant was performed on a reversed-phase Acquity UPLC BEH C18 column (Waters Corporation, Milford, MA) using an Agilent 1200 Series Rapid Resolution LC (liquid chromatography) system (Agilent Technologies, Santa Clara, CA). The LC system was coupled to an Agilent 6510 Q-TOF MS (mass spectrometer) equipped with an electrospray ionization source. Subsequent mass spectrometric analysis has been previously described (1).

Agilent GeneSpring MS version 1.2 software was used to analyze datasets in numerical data matrices. Principal components analysis was applied for differentiation of subjects with various pathophysiological features. The relative concentrations of metabolites in separate groups were compared by ANOVA with Tukey honestly significant difference correction using SPSS 13.0 (International Business Machines Corporation, Chicago, IL) software. Accurate masses of features showing significant differences between groups were searched against the METLIN Metabolite Database, the Human Metabolome Database, and the Kyoto Encyclopedia of Genes and Genomes database.

For confirmation of metabolite identity, samples were run under chromatographic conditions similar to those of profiling studies. Mass spectrometric analyses were performed using the Agilent 6510 Q-TOF MS under similar conditions.

RESULTS

—As shown in Table 1, the subjects' average BMI, blood glucose level, blood pressure, and lipid levels were just slightly above the standard values, reflecting the typical characteristics of patients with metabolic syndrome. Through urinary data analysis, metabolites were grouped according to BMI, hypertension, diabetes, total cholesterol (TC), and triacylglycerol (TG) indexes. We observed that many metabolites (e.g., nicotinuric acid, hypoxanthine, tiglylcarnitine, 1-methylhistidine) were the basis for clustering of certain indexes (BMI, TC, TG and blood pressure before eating, and having or not having diabetes), but only nicotinuric acid clustered all indexes.

The level of urine nicotinuric acid in subjects who were overweight (BMI ≥ 24 kg/m^2) (n = 40) was higher than in those who were not (BMI < 24 kg/m^2)

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Table 1—*Baseline clinical and biochemical characteristics of subjects*

	Value
Participants, *n*	99
Age (y)	69 (13)
Women	66 (66.7%)
Personal history	
Smoking	21 (21.2%)
Alcohol consumption	28 (28.3%)
Medical history	
Hypertension	64 (64.6%)
Diabetes	33 (33.3%)
Examination	
BMI (kg/m^2)	25.8 (4.1)
Systolic BP (mmHg)	144.2 (18.3)
Diastolic BP (mmHg)	82.7 (11.3)
Investigations	
HbA_{1c} (%)	7.0 (1.9)
TC (mg/dL)	220.2 (46.9)
LDL-C (mg/dL)	117.9 (40.2)
HDL-C (mg/dL)	49.9 (15.9)
TG (mg/dL)	179.9 (45.6)
hs-CRP (mg/dL)	1.0 (2.5)

Data are mean (SD) or *n* (%), unless otherwise indicated. BP, blood pressure.

(n = 23) (221 ± 23 vs. 144 ± 18 × 10^3 milliabsorbance units [mAU], P = 0.0265) and was higher in subjects with high blood pressure (≥140/90 mmHg) (n = 40) than in normotensive subjects (n = 28) (238 ± 28 vs. 154 ± 19 × 10^3 mAU, P = 0.0269). In subjects with diabetes (glycated hemoglobin [HbA_{1c}] ≥6.5% or receiving diabetes medication) (n = 25), urine nicotinuric acid level was higher than in those without diabetes (n = 37) (221 ± 31 vs. 152 ± 13 × 10^3 mAU, P = 0.0268), and in subjects with high levels of TG (≥150 mg/dL) (n = 44), it was higher than in those with normal levels of TG (<150 mg/dL) (n = 21) (218 ± 20 vs. 136 ± 22 × 10^3 mAU, P = 0.0147). In subjects with high levels of LDL cholesterol (LDL-C) (≥100 mg/dL) (n = 37), urine nicotinuric acid values were higher than in those with normal levels of LDL-C (<100 mg/dL) (n = 18) (239 ± 26 vs. 158 ± 20 × 10^3 mAU, P = 0.0426), and it was higher in subjects with high TC levels (≥200 mg/dL) than in those with normal levels (data not shown). On the other hand, urine nicotinuric acid values in subjects with low levels of HDL cholesterol (HDL-C) (<40 mg/dL) (n = 14) were higher than in those with normal levels of HDL-C (≥40 mg/dL) (n = 36) (234 ± 23 vs. 167 ± 14 × 10^3 mAU, P = 0.0134).

Moreover, we selected high-sensitivity C-reactive protein (hs-CRP) as the index for assessing cardiovascular disease risk. Urine nicotinuric acid values in subjects with high levels of hs-CRP (≥0.3 mg/dL) (n = 23) were higher than in those with low levels of hs-CRP (<0.3 mg/dL) (n = 32) (252 ± 37 vs. 170 ± 15 × 10^3 mAU, P = 0.0296).

CONCLUSIONS—To our knowledge, this report is the first to show that urinary nicotinuric acid level is positively correlated with BMI, blood pressure, and plasma HbA_{1c}, TC, LDL-C, TG, and hs-CRP levels but negatively correlated with HDL-C level. Nicotinuric acid is the major catabolic product of nicotinic acid and regarded as a good index for assessing nicotinic acid biotransformation in the liver (2). The structure of nicotinuric acid is a type of acylglycine, which participates in various coenzyme tasks, including glycolysis, gluconeogenesis, the citric acid cycle, and oxidation, for all tissues as well as in forming long-chain fatty acids (3). Previous studies indicated that acylglycine in urine reflects an accumulating condition of acyl-CoA ester in mitochondria (4). Moreover, long-chain acyl-CoA esters have been addressed as an index for muscle lipid metabolism and negatively correlated to the effects of insulin (5).

Nicotinic acid is transformed from tryptophan and mainly used to produce two coenzymes, NAD^+ and $NADP^+$ (6). A recent study revealed that 5-hydroxyindole-3-acetic acid (a derivative end product of serotonin converted from tryptophan) concentrations are high in subjects with metabolic syndrome (7). Mechanistically, $NADP^+$ and NADPH are the coenzymes for the oxidation-reduction reactions of fatty acid synthesis (8,9). Nicotinic acid has been proven to effectively reduce TG and LDL-C levels and increase HDL-C levels, but it increases the possibility of insulin resistance (10,11). In this respect, nicotinuric acid in urine reasonably shows the changes of lipid metabolism and insulin resistance, which comprise the core of the pathological mechanism of metabolic syndrome.

Recent studies showed that the concentration of plasma choline is related to cardiovascular disease risk (12,13). Betaine and glycine are part of the metabolic pathways for choline (14). In this regard, the metabolites of nicotinic acid (nicotinuric acid [*N*-nicotinoyl-glycine] and trigonelline [betaine nicotinate]) could also reflect part of the metabolism of choline (Supplementary Fig. 1).

In conclusion, this study proposes that nicotinuric acid could be used to represent a potential pathogenic mechanism of metabolic syndrome. However, there are two limitations that need to be addressed. First, the subjects did not fully meet the diagnostic criteria for metabolic syndrome, although comparative parameters included in this study are much wider than the current diagnostic criteria for metabolic syndrome. Second, the presence of atherosclerotic cardiovascular disease was not confirmed in the subjects but only speculated by serum hs-CRP level. Regardless, these findings bring a new perspective not only to the lipid metabolism and insulin resistance properties of nicotinuric acid, but also to the balance of NAD^+/NADH and $NADP^+$/NADPH, and deserve further exploration.

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C.-F.H. acquired, analyzed, and interpreted data and wrote, critically revised, and approved the manuscript. M.-L.C. performed the statistical analysis and critically revised and approved the manuscript. C.-M.F. provided technical support and critically revised and approved the manuscript. C.-Y.H. performed the statistical analysis, provided technical support, and critically revised and approved the manuscript. M.-S.S. obtained funding; conceived of, directed, and supervised the study; acquired, analyzed, and interpreted the data; and critically revised and approved the manuscript. C.-F.H. and M.-S.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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