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Association of a Tobacco-specific Nitrosamine Carcinogen with Urinary Cotinine, Urinary Sodium Excretion, and Total Energy Intake in Adolescents and Children*

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Summary: This study investigated the association of a tobacco-specific nitrosamine carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) with urinary cotinine (uCot), urinary sodium (uNa) excretion, systolic blood pressure (sBP), and total energy intake in adolescents and children in relation to the subjects' age. A total of 790 subjects aged 6–19 years were evaluated. NNAL, uCot, corrected NNAL (cNNAL), the NNAL/uCot ratio, uNa, sBP, and nutrient intake were measured. A strong association between uCot and cNNAL was observed in children who were 11 years of age (r=0.881, P<0.001); however, no significant association was noted in adolescents who were 19 years of age. The uNa level was significantly higher (133.9 mmol/L *vs.* 107.8 mmol/L, P<0.001) and sBP was significantly lower (105.3 mmHg *vs.* 110.6 mmHg, P=0.012) in adolescents with elevated NNAL than in those without elevated NNAL. NNAL was significantly higher in subjects with increased uNa excretion than in those without increased uNa excretion. NNAL was positively correlated with uNa (r=0.183, P<0.001) and negatively correlated with sBP (r=–0.142, P<0.001). Non-smokers with elevated NNAL/uCot ratios had significantly lower total energy intake than those without elevated NNAL/uCot ratios (1729.0 kcal/day *vs.* 1911.0 kcal/day, P=0.008). The relationship between NNAL and uCot varied according to the subjects' age. NNAL seems to play a role in decreasing sBP by enhancing uNa excretion. Insufficient nutrient intake may contribute to endogenous formation of NNAL in non-smoking adolescents and children.
Key words: nitrosamine; cotinine; urinary sodium excretion; nutrient intake; blood pressure

A metabolite of the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), is a potent lung carcinogen[1]. NNAL originates from N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) that are produced via nitrosation of nicotine[2]. As NNK is rapidly metabolized into NNAL, and NNK is not detectable in urine, NNAL has been widely used as a reliable indicator for the uptake of tobacco-specific carcinogens[3].

Cotinine is the major metabolite of nicotine and regarded as a sensitive biomarker for determining direct and passive smoking. Nicotine has a short half-life of forty minutes; however, cotinine has a longer half-life of 16–18 hours[4, 5]. Furthermore, cotinine can be detected in urine for three days after cigarette smoking[6]. Therefore, cotinine is more helpful for determining smoking status than nicotine. In particular, self-reports of non-smoking and confirmation of smoking cessation can be biochemically verified by measuring cotinine.

The initiation of smoking usually occurs in adolescence; almost 90% of those who become long-term smokers start smoking during adolescence[7]. Secondhand smoke (SHS) is defined as the smoke exhaled by smokers and the smoke from the burning tip of a cigarette. Sidestream smoke is approximately four times more toxic than mainstream smoke because sidestream smoke is directly emitted without filtering[8]. Nearly 25% of non-smokers are still exposed to SHS[9]. In childhood, exposure to SHS from parental smoking at home is a serious public health problem.

There have been extensive studies on the associations between cigarette smoking, urinary cotinine (uCot), and NNAL in adult smokers. However, few studies have closely examined the relationship

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between uCot and corrected NNAL (cNNAL) in adolescents and children, particularly in conjunction with urinary sodium (uNa) excretion, systolic blood pressure (sBP), and nutrient intake. Here, we investigated the relationship between smoking-related biomarkers, uNa, sBP, and total energy intake in adolescents and children. We also investigated the characteristics of non-smoking subjects who had elevated NNAL/uCot ratio.

1 MATERIALS AND METHODS

1.1 Study Population

A total of 790 subjects including 260 adolescents and 530 children were evaluated. Data were obtained from the Korea National Health and Nutrition Examination Survey-2017 (KNHANES-2017), which is a nationwide representative study conducted by the Korean Ministry of Health and Welfare. The present cross-sectional analysis was restricted to participants aged 6 to 19 years who completed the health examination survey, including uCot, NNAL, smoking history, complete blood cell counts, biochemical profiles, anthropometric measurements, and nutrient intake (n=814). We excluded the following subjects: (a) those who had abnormal kidney and liver function (to avoid the potential impact on nicotine metabolism), (b) those with current usage of medications, (c) those who had smoking cessation treatment or electronic cigarette smoking, and (d) those who had missing values for study variables. Thus, the final sample consisted of 408 male and 382 female participants. The study protocol was approved by the institutional review board of Inha University Hospital (approval number: 2020-06-003-000).

1.2 Smoking Status

Information about smoking status was collected using a questionnaire during the health interview survey. Smoking history included average number of smoked cigarettes per day, days of smoking in the past month, the characteristics of smoking behaviors, and the presence of participation in smoking cession therapy. The smoking status was validated by the uCot level. A uCot level of 15 ng/mL was used as the cutoff level for the exposure to cigarette smoke, and individuals with uCot>50 ng/mL were defined as cotinine-verified smokers[10]. Among the participants without past history of smoking, subjects who had uCot levels (15–50 ng/mL) were included in an SHS exposure group.

1.3 Measurement of uCot and NNAL

Fresh urine samples were collected and immediately tested for routine urinalysis, and the remaining aliquots were stored at –20°C until analysis of uCot and NNAL. Negative controls were simultaneously analyzed with each set of urine samples. Urine concentrations of cotinine and total NNAL (free NNAL plus NNAL-glucuronide) were analyzed by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) using Agilent 1100 Series API 4000 (AB SCIEX, USA) and Agilent 1200 Series Triple Quadrupole 5500 (AB SCIEX), respectively, as described previously[11]. The limits of detection were 0.27 ng/mL for uCot and 0.02 pg/mL for NNAL.

1.4 Calculation of cNNAL and NNAL/uCot Ratio

NNAL was normalized to urinary creatinine concentration, and the creatinine-normalized NNAL was defined as cNNAL, which was calculated using the following equation: cNNAL (pg/mg) = NNAL (pg/mL)/urinary creatinine (mg/mL). The NNAL/uCot ratio was estimated with the following formula: NNAL/uCot ratio ($\times 10^{-3}$) = NNAL (pg/mL)/uCot (ng/mL).

1.5 Clinical and Laboratory Measurements

Clinical and laboratory parameters, such as blood pressure, body mass index (BMI), urinary sodium and potassium excretion, serum creatinine level, and hepatic enzyme activity were measured. Measurements of complete blood cell counts, biochemical profiles, and urinalysis were conducted with the Sysmex XN-9000 (Sysmex, Japan), Hitachi automatic analyzer 7600-210 (Hitachi, Japan), and Urisys 2400 (Roche, Germany), respectively. Urinary creatinine level was measured using a Creatinine-HR 1-Type Wako (WAKO, Japan). High-sensitivity C-reactive protein (hsCRP) was measured by immunoturbidimetry using the Cobas 8000 (Roche, Germany).

1.6 Nutrient Intake

Information about the daily food intake was obtained from the nutrition survey, including total energy intake, proteins, lipids, carbohydrates, and sodium and potassium intake. The questionnaire, which was administered by a trained dietician, was used to collect data on food items. A 24-hour dietary recall method was used as a dietary assessment tool, which is a structured interview for obtaining information about all foods consumed in the past 24 hours, including food name, ingredients, and amount of intake. Because food substances are ingested as a combination of various foods, the type and amount of food items were converted to the units of nutrients and total calories based on the reference food composition tables[12].

1.7 Categorization of Subjects

To strictly compare NNAL levels between smokers and age-matched non-smokers, subjects aged 14–19 years were classified as adolescents. Subjects were categorized into two groups based on the uCot level: smokers (uCot>50 ng/mL; n=35) and non-smokers (uCot<15 ng/mL; n=755). Subjects were classified into two groups based on the levels of NNAL and the NNAL/uCot ratio: adolescents with NNAL>1.26 pg/mL (n=112) and NNAL≤1.26 pg/mL

(n=113); non-smokers with NNAL/uCot ratio>3.01 ($\times10^{-3}$; n=377) and NNAL/uCot ratio≤3.01 ($\times10^{-3}$; n=378). These figures were provisional cutoff limits based on the median levels of NNAL and the NNAL/uCot ratio of the corresponding subjects. To assess the association between NNAL and uNa excretion, non-smoking adolescents were further stratified into each of the two groups based on the mean and quartile levels of uNa: subjects with uNa>120.3 mmol/L (n=112) and uNa≤120.3 mmol/L (n=113); subjects with uNa>156.0 mmol/L (upper 25th percentile; n=56) and uNa<84.0 mmol/L (lower 25th percentile; n=56).

1.8 Statistical Analysis

To analyze the difference of mean between the two groups, a Student's t-test and a Mann-Whitney U test were used. The normality of data distribution was confirmed by a Kolmogorov-Smirnov's one-sample test. Data were analyzed using the survey sample weights that were assigned to each sample person to generate a nationally representative estimate. Data were expressed as the weighted mean±standard error, weighted median (interquartile range), or frequency (percentage). The associations between uCot, NNAL, and cNNAL were assessed by a multivariate regression analysis after adjusting for potential confounders, including age, gender, body mass index (BMI), sBP, alanine aminotransferase (ALT), hsCRP, and total energy intake. To create scatter plots, cNNAL levels were log-transformed because their distributions were skewed. The data were analyzed by using SPSS software (IBM SPSS Statistics for Windows, Version 19.0., USA). A value of P<0.05 was considered statistically significant.

2 RESULTS

2.1 Baseline Characteristics of Subjects

Of the 790 subjects, 35 (4.4%) were uCot-verified smokers, whereas 21 (2.6%) were self-reported smokers. In smokers, the mean duration of smoking was 2.8 years, the number of cigarettes smoked was 7.6 per day, and the age at onset of smoking was 15.1 years of age. Among the total subjects, 53 (6.7%) had a history of exposure to SHS. The incidence of being underweight was 3.4%, whereas the incidence of being overweight was 12.1%, which was based on the Korean growth standards according to age and gender. Elevated hsCRP level, hypertension, and anemia were observed in 21.6%, 10.5%, and 2.0% of the subjects, respectively (table 1).

2.2 Smokers *vs.* Non-smokers

The uCot-verified smokers were compared with age- and gender- matched non-smokers. The median uCot, NNAL, and cNNAL levels were significantly

Table 1 Baseline characteristics and smoking status of subjects

	Frequency	Proportion (%)
Total subjects (n=790)		
Children (6–13 years)	530	67.1
Adolescents (14–19 years)	260	32.9
Gender (male)	408	51.6
Smoking status (uCot level)		
uCot-verified smokers (>50 ng/mL)	35	4.4
uCot-verified non-smokers (<15 ng/mL)	755	95.6
Duration of smoking (years)	2.8±0.13	NA
Numbers of cigarette smoking (/day)	7.6±0.15	NA
Onset of smoking (years)	15.1±0.24	NA
Self-reported smokers	21	2.6
SHS exposure	53	6.7
Clinical and laboratory parameters		
Overweight	96	12.1
Underweight	27	3.4
hsCRP >0.5 mg/dL	171	21.6
Leukocyte >11000/μL	4	0.5
Anemia (n)	16	2.0
Hypertension (n)	83	10.5
Nutrient intake		
Total energy intake (kcal/day)	1814.5 (1357.2–2319.7)	NA
Proteins (g/day)	65.0 (45.0–83.9)	NA
Lipids (g/day)	49.5 (32.0–71.0)	NA
Carbohydrates (g/day)	281.0 (215.0–352.0)	NA
Na intake (mg/day)	2601.5 (1712.8–3650.5)	NA
K intake (mg/day)	2165.0 (1539.2–2821.5)	NA

Data are expressed as the weighted mean±standard error, weighted median (interquartile range), or frequency (percentage). uCot: urinary cotinine; SHS: secondhand smoke; BMI: body mass index; hsCRP: high sensitivity C-reactive protein; NA: not applicable

higher in smokers than in non-smokers; however, the NNAL/uCot ratio was significantly higher in non-smokers than in smokers. There were no significant differences in the levels of hemoglobin, liver and kidney function, and inflammatory parameters between smokers and non-smokers (table 2).

2.3 NNAL and Subjects' Age

The NNAL concentrations in adolescents and children according to the subjects' age were evaluated. As shown in fig. 1, young children and adolescents who were 8 and 19 years of age, respectively, had high NNAL concentrations compared to the subjects of other age groups (fig. 1).

2.4 uNa and sBP According to NNAL

Excretion of uNa was significantly higher in adolescents with elevated NNAL than in those without elevated NNAL (133.9 mmol/L *vs.* 107.8 mmol/L, $P<0.001$). However, there was no significant difference in dietary sodium intake between the two groups. Subjects with elevated NNAL had significantly lower sBP than those without elevated NNAL (105.3 mmHg *vs.* 110.6 mmHg, $P=0.012$) (table 3).

2.5 Association of NNAL with uNa Excretion

NNAL level was significantly higher in subjects with increased uNa excretion>120.3 mmol/L than those without increased uNa excretion≤120.3 mmol/L (1.57 pg/mL *vs.* 1.22 pg/mL, $P=0.031$). NNAL level was much higher in subjects with uNa excretion>156.0 mmol/L (upper 25th percentile) than those with uNa excretion<84.0 mmol/L (lower 25th percentile) (1.59 pg/mL *vs.* 0.92 pg/mL; $P<0.001$). However, there were no significant differences in uCot level between the subjects with uNa>120.3 mmol/L and those with uNa≤120.3 mmol/L, nor between the subjects with uNa>156.0 mmol/L and those with uNa<84.0 mmol/L (table 4).

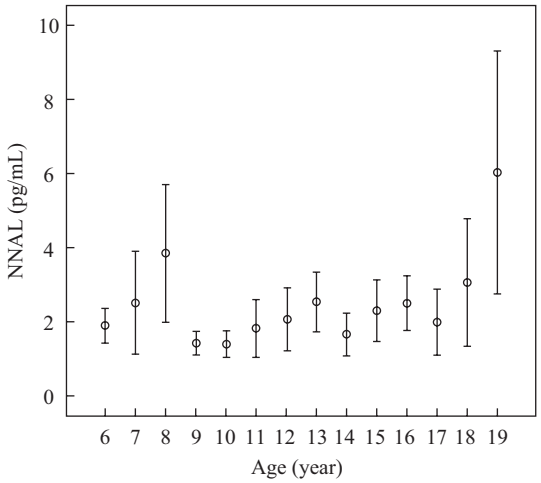

Fig. 1 The distribution of urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) concentrations in relation to the subjects' age.
Urinary NNAL concentrations were higher in young children and adolescents who were 8 and 19 years of age, respectively, compared to other age groups.

2.6 NNAL/uCot Ratio and Nutrient Intake

Subjects with elevated NNAL/uCot ratios had significantly lower total energy intake than those without elevated NNAL/uCot ratios (1729.0 kcal/day

Table 2 Smoking-related biomarkers and laboratory parameters in smokers and non-smokers

	Smokers (uCot>50 ng/mL; *n*=35)	Non-smokers (age- and gender-matched; uCot<15 ng/mL; *n*=105)	*P* value
Gender (male; *n*)	24 (68.6)	75 (71.4)	0.721
Age (year)	17.7±0.12	17.3±0.13	0.146
BMI (kg/m^2)	22.1±0.06	21.9±0.04	0.762
sBP (mmHg)	111.3±1.08	112.1±1.02	0.683
Heart rate (/min)	72.3±0.41	73.1±0.45	0.095
Smoking-related biomarkers			
uCot (ng/mL)	774.0 (391.0–1288.0)	0.58 (0.38–0.83)	<0.001
NNAL (pg/mL)	64.9 (51.2–162.0)	2.17 (0.74–5.07)	<0.001
cNNAL (pg/mg)	28.5 (18.1–70.8)	0.87 (0.37–1.89)	<0.001
NNAL/uCot ratio (×10^{-3})	0.12 (0.08–0.14)	3.56 (1.79–5.82)	<0.001
Laboratory parameters			
Hemoglobin (g/dL)	15.3±0.14	15.1±0.12	0.431
Leukocyte (×10^3/μL)	6.95±0.28	6.52±0.23	0.175
AST (IU/L)	17.4±0.32	18.3±0.34	0.285
ALT (IU/L)	13.2±0.29	14.7±0.27	0.332
Serum creatinine (mg/dL)	0.86±0.03	0.83±0.02	0.265
hsCRP (mg/dL)	0.60 (0.35–1.70)	0.44 (0.32–0.78)	0.412
hsCRP >0.5 mg/dL (*n*)	11 (31.4)	39 (37.1)	0.543

Data are expressed as the weighted mean±standard error, weighted median (interquartile range), or frequency (percentage). Student's *t*-test or Mann-Whitney *U* test was used.

BMI: body mass index; sBP: systolic blood pressure; uCot: urinary cotinine; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; cNNAL: corrected NNAL; NNAL/uCot ratio: the ratio of NNAL to uCot; AST: aspartate aminotransferase; ALT: alanine aminotransferase; hsCRP: high-sensitivity C-reactive protein

Table 3 uNa excretion and sBP in subjects with and without elevated NNAL level

	Non-smoking adolescents (n=225)		
	Elevated NNAL>1.26 pg/mL (n=112)	Non-elevated NNAL≤1.26 pg/mL (n=113)	P value
Anthropometric parameters			
Gender (male; n, %)	60 (53.5)	48 (42.5)	0.097
Age (year)	16.4±0.15	16.1±0.14	0.135
BMI (kg/m^2)	20.4±0.06	21.3±0.05	0.031
sBP (mmHg)	105.3±1.24	110.6±1.19	0.012
dBP (mmHg)	68.1±0.72	69.2±0.65	0.332
Urine electrolytes			
uNa (mmol/L)	133.9±0.27	107.8±0.23	<0.001
uK (mmol/L)	50.2±0.14	38.1±0.12	<0.001
Nutrient intake			
Na intake (mg/day)	3111.0 (2210.0–4537.0)	3150.0 (1872.0–4653.0)	0.512
K intake (mg/day)	2211.0 (1607.0–3263.0)	2291.0 (1575.0–3103.0)	0.237
Smoking-related biomarkers			
uCot (ng/mL)	0.58 (0.33–0.89)	0.34 (0.27–0.47)	<0.001
NNAL (pg/mL)	3.26 (1.65–5.58)	0.65 (0.46–0.90)	<0.001
NNAL/uCot ratio (×10^{-3})	5.41 (4.32–6.56)	1.79 (1.17–2.67)	<0.001
cNNAL (pg/mg)	1.17 (0.74–2.21)	0.38 (0.25–0.54)	<0.001

Data are expressed as the weighted mean±standard error, weighted median (interquartile range), or frequency (percentage). Student's t-test or Mann-Whitney U test was used.
BMI: body mass index; sBP: systolic blood pressure; dBP: diastolic blood pressure; uNa: urinary sodium; uK: urinary potassium; uCot: urinary cotinine; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL/uCot ratio: the ratio of NNAL to uCot; cNNAL: corrected NNAL

Table 4 NNAL and uCot levels according to uNa excretion in non-smoking adolescents

	NNAL (pg/mL)	P value	uCot (ng/mL)	P value
Mean level of uNa				
>120.3 mmol/L (n=112)	1.57 (0.89–3.47)		0.41 (0.27–0.78)	
≤120.3 mmol/L (n=113)	1.22 (0.63–2.26)	0.031	0.36 (0.27–0.64)	0.205
Upper and lower 25th percentile of uNa				
>156.0 mmol/L (n=56)	1.59 (0.95–4.50)		0.42 (0.27–0.92)	
<84.0 mmol/L (n=56)	0.92 (0.58–1.77)	<0.001	0.34 (0.27–0.58)	0.083

Data are expressed as the weighted median (interquartile range). Mann-Whitney U test was used.
uNa: urinary sodium; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; uCot: urinary cotinine

vs. 1911.0 kcal/day, P=0.008). Serum total cholesterol and fasting plasma glucose levels were significantly lower in subjects with elevated NNAL/uCot ratios than in those without elevated NNAL/uCot ratios. NNAL was 3.2-fold higher in subjects with a NNAL/uCot ratio>3.01 than in those with a NNAL/uCot ratio≤3.01. However, no significant difference was observed in uCot levels between the two groups (table 5).

2.7 Correlation between uCot and cNNAL

After adjusting for potential confounders, the correlation between uCot and cNNAL was evaluated according to the subjects' age. A strong association between uCot and cNNAL was observed in children and adolescents who were 11 (r=0.881, P<0.001) and 17 (r=0.863, P<0.001) years of age, respectively. However, there was no significant association between the two biomarkers in adolescents who were 19 years of age (table 6).

2.8 Regression Analysis of NNAL, sBP, and uNa

Relationship between smoking-related biomarkers, sBP, and uNa excretion in non-smokers were evaluated using multivariate linear regression analysis. NNAL was positively correlated with uNa (r=0.183, P<0.001) and negatively correlated with sBP (r=–0.142, P<0.001). The sBP was more closely associated with NNAL than uCot (table 7). Scatter plots showing the relationship between sBP, uNa, and log cNNAL levels are presented in fig. 2.

3 DISCUSSION

In the present study, urinary NNAL concentrations in adolescents and children were evaluated according to the subjects' age. NNAL concentrations were higher in young children and adolescents who were 8 and 19 years of age, respectively, than in other age groups. Our results were in accordance with the results of a previous study, which demonstrated that NNAL levels in children of 6–11 years old were approximately four times higher than those of adults[13]. These results suggest that the risk faced by non-smokers varies according to age, and children and adolescents who are

Table 5 Total energy intake in subjects with and without elevated NNAL/uCot ratio

	NNAL/uCot ratio in non-smokers (n=755)		
	>3.01 ($\times10^{-3}$; n=377)	≤ 3.01 ($\times10^{-3}$; n=378)	P value
Gender (male; n, %)	194 (51.4)	186 (49.2)	0.536
Age (year)	12.0±0.18	11.6±0.15	0.143
BMI (kg/m^2)	19.1±0.06	19.2±0.04	0.697
Nutrient intake			
Total energy intake (kcal/day)	1729.0 (1329.0–2293.0)	1911.0 (1481.0–2356.0)	0.008
Proteins (g/day)	63.0 (44.0–81.0)	68.0 (49.0–84.0)	0.037
Lipids (g/day)	47.0 (29.0–67.0)	53.0 (37.0–76.0)	0.015
Carbohydrates (g/day)	271.0 (209.0–344.0)	293.0 (225.0–372.0)	0.011
Na intake (mg/day)	2598.0 (1698.0–3614.0)	2610.0 (1783.0–4022.0)	0.913
K intake (mg/day)	2039.0 (1474.0–2768.0)	2291.0 (1684.0–3039.0)	0.002
Biochemical profiles			
Total cholesterol (mg/dL)	161.2±0.72	170.6±0.65	<0.001
Plasma glucose (mg/dL)	90.6±0.41	92.8±0.35	<0.001
HbA1c (%)	5.31±0.13	5.35±0.12	0.112
Smoking-related biomarkers			
uCot (ng/mL)	0.33 (0.27–0.56)	0.37 (0.27–0.63)	0.079
NNAL (pg/mL)	1.98 (1.28–3.65)	0.61 (0.40–0.86)	<0.001
NNAL/uCot ratio ($\times10^{-3}$)	4.90 (3.65–6.55)	1.56 (1.12–2.28)	<0.001
cNNAL (pg/mg)	1.22 (0.77–1.89)	0.54 (0.34–0.87)	<0.001

Data are expressed as the weighted mean±standard error, weighted median (interquartile range), or frequency (percentage). Student's t-test or Mann-Whitney U test was used.

BMI: body mass index; Na: sodium; K: potassium; HbA1c: hemoglobin A1c; uCot: urinary cotinine; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL/uCot ratio: the ratio of NNAL to uCot; cNNAL: corrected NNAL

Table 6 Relationship between uCot and cNNAL in non-smokers in relation to the subjects' age

Age (year)	Numbers of subjects (n=755)	Multivariate regression analysis of uCot and cNNAL*	
		Standardized β	P value
Children (total; 6–13)	530	0.548	<0.001
6	76	0.317	0.006
7	79	0.572	<0.001
8	66	0.765	<0.001
9	77	0.223	0.049
10	70	0.235	0.042
11	47	0.881	<0.001
12	53	0.465	<0.001
13	62	0.504	<0.001
Adolescents (total; 14–19)	225	0.580	<0.001
14	36	0.632	<0.001
15	45	0.789	<0.001
16	44	0.531	<0.001
17	51	0.863	<0.001
18	29	0.472	0.009
19	20	0.165	0.502

*: Adjusted for gender, BMI, sBP, ALT, hsCRP, and total energy intake. uCot: urinary cotinine; cNNAL: corrected NNAL

Table 7 Multivariate regression analysis of smoking-related biomarkers, sBP, and uNa in non-smokers

	Multivariate regression analysis*	
	uCot (ng/mL)	NNAL (pg/mL)
Subjects (n=755)		
sBP (mmHg)	–0.051 (0.293)	–0.142 (<0.001)
uNa (mmol/L)	0.102 (0.030)	0.183 (<0.001)
uK (mmol/L)	0.029 (0.517)	0.065 (0.174)

Data are expressed as standardized β (P value).

*: Adjusted for age, gender, BMI, sBP, ALT, hsCRP, and total energy intake. sBP: systolic blood pressure; uNa: urinary sodium; uK: urinary potassium; uCot: urinary cotinine; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; cNNAL: corrected NNAL

8 and 19 years of age are the most vulnerable groups, based on NNAL concentration. These findings may reflect the severity of exposure to SHS of smoking family members at home.

Prolonged cigarette smoking induces an increase in hemoglobin concentrations[14]. However, in our study, there was no significant difference in hemoglobin concentrations between smokers and non-smokers. These inconsistencies are presumably due to the duration of smoking history in our subjects. In the present study, a mean duration of smoking of only 3.3 years was observed in smokers, which may be too short for smoking to have any effect on blood hemoglobin level.

Cigarette smoking is associated with a greater risk for developing chronic obstructive pulmonary disease, asthma, atherosclerosis, and cardiovascular diseases[15]. However, the association between cigarette smoking and hypertension remains unclear. There have been discordant results on the relationship between smoking and hypertension. Some studies have reported that cigarette smoking increases BP due to

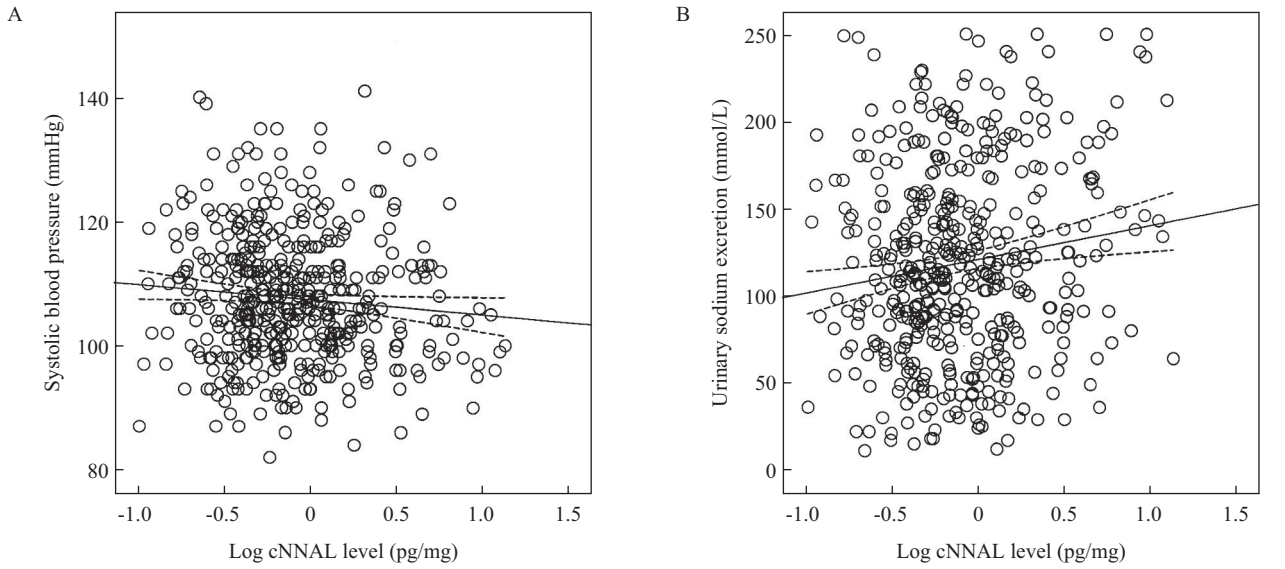

Fig. 2 Scatter plots showing the relationship between corrected 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (cNNAL) and levels of systolic blood pressure (A) and urinary sodium excretion (B) in non-smoking subjects. The log cNNAL levels were inversely correlated with systolic blood pressure (y=–2.46x+107.4; r=–0.098, P=0.031) and positively correlated with urinary sodium excretion (y=19.43x+120.7; r=0.141, P=0.002).

the effect of nicotine on the activation of sympathetic nervous system[16, 17]. In contrast, other studies have reported the effect of nicotine on decrease in BP via relaxation of vascular smooth muscle[18, 19]. In our study, subjects with elevated NNAL had significantly lower sBP than those without elevated NNAL. Multivariate regression analysis revealed that NNAL was inversely associated with sBP. Our results were consistent with the previous results that smoking was negatively correlated with systolic and diastolic BP, and that new and sustained smoking decreased the risk for incident hypertension[20, 21]. However, our results were inconsistent with the results showing that serum cotinine levels were positively associated with hypertension and that SHS exposure was significantly associated with hypertension in non-smoking women[22, 23]. These discrepancies may be derived from the individual differences in the sensitivity of nicotine and its metabolites to the sympathetic nervous system and vascular smooth muscle. In our study, sBP was more closely associated with NNAL than uCot, suggesting that NNAL plays more important roles in sBP than does cotinine.

Measurements of 24-hour uNa excretion have been commonly used for estimating dietary sodium intake. In a previous study, uNa excretion was significantly higher in smokers than in non-smokers[24]. These findings may be attributable to an increased intake of salt in smokers or to a possible natriuretic effect of the smoking-related chemicals. In our study, the effect of NNAL on uNa excretion was evaluated. The uNa level was significantly higher in subjects with elevated NNAL than in those without elevated NNAL, although there was no significant difference in dietary sodium intake between the groups. Furthermore, subjects with increased uNa had significantly higher NNAL levels than those with decreased uNa. Additionally, NNAL was significantly associated with uNa levels following an adjustment of potential confounders. These results suggest that NNAL may contribute to elevated uNa excretion, irrespective of dietary sodium intake. It is believed that elevated NNAL may play a role in decreasing BP by enhancing uNa excretion. Our findings supported the results showing that the prevalence of incident hypertension was lower in subjects with high uNa excretion than in those with low uNa excretion[25].

Smokers generally have a lower BMI than non-smokers. Cessation of smoking leads to weight gain in smokers[26]. After quitting smoking, 33% to 75% of former smokers experienced a weight gain of approximately 4.4–5.0 kg[27, 28]. There are two hypotheses explaining for the effect of smoking on BMI. First, smoking increases energy expenditure owing to nicotine's effect on enhancing metabolic rate[29]. For instance, energy expenditure is increased by 140–200 kcal/day in smokers than in non-smokers[30]. Second, smoking alters energy intake by inducing an anorexic effect[31], and smoking leads to taste impairment by dulling the perception of taste[32]. In our study, smokers showed a propensity toward decrease in BMI compared with non-smokers; however, the findings did not reach a statistical significance, probably due to the small sample size of smokers and the short duration of smoking.

To assess the characteristics of the subjects with

elevated NNAL level who had no elevation of uCot level, individuals with elevated NNAL/uCot ratios was compared to those without elevated NNAL/uCot ratios. Subjects with elevated NNAL/uCot ratios had significantly lower total calorie intake than those without elevated NNAL/uCot ratios. Moreover, serum total cholesterol concentration and fasting plasma glucose level were significantly lower in subjects with elevated NNAL/uCot ratios than in those without elevated NNAL/uCot ratios. These results suggest that insufficient nutrient intake may lead to aberrant increase in NNAL in our study populations.

The cNNAL is known to be more accurate than NNAL because the value was adjusted for urinary creatinine level[33]. In the present study, the association between cNNAL and uCot was assessed in relation to the subjects' age. There was a strong association between cNNAL and uCo in children who were 11 years of age, whereas no significant association between the biomarkers was found in adolescents who were 19 years of age. These results may reflect the difference in the formation of NNAL between children and adolescents. Previous studies have reported that nitrosamine carcinogen is detected in the saliva of non-smokers, which may be linked to oral bacteria and poor oral hygiene[34], and that nitrosamine can be formed through the reduction of dietary nitrate, particularly in the acidic conditions of the stomach[35]. In our study, the NNAL level was 3.2 times higher in subjects with elevated NNAL/uCot ratios than in those without elevated NNAL/uCot ratios, although no significant difference in uCot level was noted between the groups. Interestingly, in the present study, non-smokers had significantly higher NNAL/uCot ratios than did smokers. Based on these findings, it is estimated that NNAL may form endogenously in some individuals, irrespective of uCot level, which may account for our results on the non-significant association between cNNAL and uCot in the adolescents who were 19 years of age.

There are several limitations in this study. As the present study is a cross-sectional design, the causal effect of NNAL on uNa excretion and sBP was not clarified in this study. We did not use 24-hour urine samples, but rather random spot urine samples that were collected at specific time point. Effect of smoking-related variables on NNAL was not assessed due to the small sample size of self-reported smokers. Despite these limitations, our study has significance. To the best of our knowledge, this is the first study to report meaningful associations between NNAL, sBP, and uNa, as well as the potential contribution of insufficient nutrient intake to enhanced NNAL production.

In conclusion, this study demonstrates that the relationship between NNAL and uCot varies according to the subjects' age. NNAL seems to form endogenously in some individuals, particularly in conjunction with insufficient total energy intake. NNAL contributes to elevated uNa excretion, irrespective of dietary sodium intake, and plays a role in decreasing sBP. Further studies are needed to validate these findings of our study in larger randomized prospective trials.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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