Serum N^1-Methylnicotinamide Is Associated With Obesity and Diabetes in Chinese

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Context: Nicotinamide *N*-methyltransferase (NNMT) is a novel histone methylation modulator that regulates energy metabolism, and NNMT knockdown prevents diet-induced obesity in mice. However, whether NNMT plays a role in human obesity and type 2 diabetes (T2DM) remains to be elucidated.

Objective: NNMT catalyzes methylation of nicotinamide to generate N^1-methylnicotinamide (me-NAM). We aimed to investigate the associations of serum me-NAM with obesity and T2DM in Chinese.

Design, Setting, and Participants: The study subjects (n = 1160) were recruited from Dali, a city of Yunnan Province, in southwest China. Anthropometric phenotypes, fasting glucose, and serum lipids were measured. Serum me-NAM was measured by liquid chromatography-mass spectrometry.

Results: Serum me-NAM was positively correlated with body mass index and waist circumference and negatively with high-density lipoprotein ($P \leq .03$). The correlations remained highly significant in the multivariate adjusted correlation analyses. In men (n = 691), positive correlations between me-NAM and fasting glucose, low-density lipoprotein, liver function, and serum creatinine levels were also observed in both simple and multivariate adjusted correlation analyses. In multiple logistic regression analyses, elevated serum me-NAM was associated with higher risks for overweight/obesity (odds ratios, 2.36 and 5.78; 95% confidence intervals, 1.10–5.08 and 1.78–18.76 for men and women, respectively; $P \leq .03$) and diabetes (odds ratios, 1.56 and 1.86; 95% confidence intervals, 1.10–2.22 and 1.05–3.31 for men and women, respectively; $P \leq .03$).

Conclusions: This first large-scale population study shows that me-NAM, as an indicator of NNMT activity, is strongly associated with obesity and diabetes, supporting NNMT as a potential target for treating obesity and diabetes in humans. **(*J Clin Endocrinol Metab* 100: 3112–3117, 2015)**

Obesity and overweight pose a major risk for the development of type 2 diabetes (T2DM), cardiovascular disease, hypertension, and stroke (1). Obesity and diabetes epidemics are not restricted to industrialized societies. In developing countries, especially those with rapid economic growth such as China and India, the obesity rate

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Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; CI, confidence interval; GGT, γ-glutamyltransferase; HDL, alanine aminotransferase; LDL, low-density lipoprotein; me-NAM, N^1-methylnicotinamide; OR, odds ratio; SAM, *S*-adenosylmethionine; NNMT, *N*-methyltransferase; T2DM, type 2 diabetes.

has increased sharply over the past 20 to 30 years (2). Environmental factors such as lifestyle changes are clearly the primary driving force for the obesity epidemic. Unmatched energy expenditure with increased energy intake is a fundamental mechanism for the development of obesity (3). Although genetic variants are important for regulating energy metabolism, they do not appear to explain the obesity prevalence in recent decades. It is increasingly recognized that epigenetic mechanisms such as DNA and histone methylation may be key in orchestrating environmental factors, gene expression, and energy metabolism in obesity development (4, 5). Because epigenetic markers are potentially reversible pharmacologically, the epigenome has become an attractive target for treating obesity and T2DM.

It was recently revealed that nicotinamide *N*-methyltransferase (NNMT) is a novel modulator of histone methylation and plays an important role in regulating cellular energy metabolism (6). NNMT is the only enzyme that catabolizes nicotinamide (vitamin B_3) by transferring a methyl group from *S*-adenosylmethionine (SAM) to nicotinamide (7, 8). The enzymatic reaction generates N^1-methylnicotinamide (me-NAM) and *S*-adenosylhomocysteine (SAH), a precursor of homocysteine (7, 8). NNMT is probably a major methyltransferase in adipose tissue because NNMT knockdown significantly increases adipose SAM levels and the SAM/SAH ratio (6). This in turn promotes the methylation of lysine 4 on histone 3. The altered histone methylation further modulates downstream target genes including the key enzymes involved in polyamine flux, thus enhancing energy consumption. Consequently, NNMT knockdown in adipose tissue and liver protects against diet-induced obesity and its deteriorating metabolic disorders (6).

A more recently study shows that adipose NNMT expression is increased in T2DM, and NNMT levels correlate positively with insulin resistance (9). More importantly, interventions that improve insulin sensitivity by exercising and bariatric surgery are associated with a significant reduction in adipose NNMT expression (9). These results suggest that NNMT may play important roles in obesity and T2DM in humans. However, measuring NNMT expression in adipose tissue and/or liver in humans has limitations and is usually not practical for epidemiological studies. me-NAM has been used as an indicator of NNMT activity because it can only be generated by NNMT. Serum and urine me-NAM can be measured using mass spectrometry (10). Both serum and urinary me-Nam levels have been shown to be elevated in obesity and T2DM in humans (9, 11). However, there are no large-scale population studies to establish the association of me-NAM with obesity and T2DM. Therefore, we measured me-NAM levels in 1160 Chinese and investigated its associations with obesity and T2DM.

Subjects and Methods

Study population

Subjects were recruited in the framework of a cross-sectional population-based study in hypertension and metabolic syndrome, as reported previously (12, 13). From March to May 2010, we invited all employees and retired workers of a factory in Dali County (25°N), Yunnan Province, China, to participate the study. Of the 1643 invited, 1443 (87.8%) participated. We excluded 283 subjects from the present analyses because of inadequate blood samples for me-NAM measurements (n = 260) or missing information on demographic data (n = 15), plasma glucose (n = 1), or serum lipids (n = 7). Thus, the total number of subjects in the present analyses was 1160. All subjects gave written informed consent. The study protocol was approved by the ethic committee of the Affiliated Hospital of Dali University.

Anthropometric and biochemical measurements

Sociodemographics, medical history, smoking and drinking habits, and the use of medications were documented with a standardized questionnaire. Body weight, body height, waist and hip circumference, and blood pressure (BP) were measured by an experienced nurse. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Three sitting BP measurements taken consecutively at 5-minute intervals using an automated electronic device (Omron HEM 7011) were averaged for analysis. Venous blood samples were taken after overnight fasting for the measurement of plasma glucose, serum creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT). Serum samples were subsequently stored in aliquots at −30°C for measurements of me-NAM.

Serum me-NAM concentrations were determined by liquid chromatography with tandem mass spectrometry (Agilent 6430 Triple Quad liquid chromatograph/mass spectrometer) with a modification of the method described by Szafarz et al (10) in the Clinical Pharmacology Laboratory, Jiangsu Province Hospital of Traditional Chinese Medicine. The intra- and interassay coefficients of variance were 3.69% and 4.95%, respectively, for me-NAM. The analytic sensitivity of the assays for measuring me-NAM was 2.5 ng/mL.

Overweight was defined as a BMI of 24 to <28 kg/m^2, and obesity was defined as a BMI of at least 28 kg/m^2 according to Chinese criteria (14). Diabetes mellitus was defined as a fasting plasma glucose of at least 7.0 mmol/L or as the use of antidiabetic agents. Hypertension was defined as a BP of at least 140 mm Hg systolic or 90 mm Hg diastolic or as the use of antihypertensive drugs.

Statistical methods

We used SAS version 9.2 (SAS Institute) for database management and statistical analyses. Data are presented as means ± SD or medians (25th and 75th percentiles) for continuous variables or as percentages for categorical variables. Departure from normality was tested by the Shapiro-Wilks statistic. Serum me-

Table 1. Characteristics of Participants

Characteristic	Men (n = 691)	Women (n = 469)	*P*
Age, y	50.5 ± 13.4	49.3 ± 12.4	.13
BMI, kg/m^2	24.0 ± 3.2	23.0 ± 3.4	<.001
Waist circumference, cm	82.2 ± 8.9	74.5 ± 9.6	<.001
Systolic BP, mm Hg	130.7 ± 19.9	125.5 ± 21.7	<.001
Current smoking, n (%)	400 (57.9)	7 (1.5)	<.001
Alcohol intake, n (%)	250 (36.2)	9 (1.9)	<.001
Overweight/obese, n (%)	339 (49.1)	162 (34.5)	<.001
Diabetes mellitus, n (%)	65 (9.4)	36 (7.7)	.30
Taking antihyperglycemic drugs, n (%)	47 (6.8)	24 (5.1)	.24
Plasma glucose, mmol/L	5.47 ± 1.78	5.30 ± 1.50	.07
Total cholesterol, mmol/L	5.50 ± 0.99	5.47 ± 1.01	.53
LDL cholesterol, mmol/L	3.29 ± 0.86	3.23 ± 0.91	.29
HDL cholesterol, mmol/L	1.25 ± 0.32	1.47 ± 0.35	<.001
Triglycerides, mmol/L	1.94 (1.28–2.92)	1.45 (1.02–2.15)	<.001
ALT, U/L	28 (20–41)	18 (14–26)	<.001
GGT, U/L	34 (22–61)	16 (11–26)	<.001
Serum creatinine, μmol/L	85.2 ± 14.7	62.5 ± 12.6	<.001
me-NAM, ng/mL	12.5 (9.0–17.6)	11.7 (8.3–15.9)	.002

Data are means ± SD, median with interquartile range in parentheses, or number with percentage in parentheses.

NAM, triglyceride, ALT, and GGT concentrations were not normally distributed and were, therefore, logarithmically transformed for statistical analyses. Means and proportions were compared with the Student *t* test and the Fisher exact test, respectively. Relationships among me-NAM, age, BMI, waist and hip circumference, BP, liver enzymes, renal function, fasting plasma glucose, and serum lipids were examined by calculation of partial correlation coefficients. We then performed multiple logistic regression analyses to evaluate the odds ratios (ORs) and 95% confidence intervals (CIs) of having overweight/obesity and diabetes for each 1-unit increase in the logarithmically transformed me-NAM. Spearman correlation and logistic regression analyses with nontransformed me-NAM were also performed. A two-sided value of $P < .05$ was considered statistically significant.

Results

Characteristics of the study participants

The 1160 participants (mean age, 50.0 ± 13.0 years) included 691 men (59.6%), 397 overweight participants (34.2%), 104 obese participants (9.0%), and 101 participants with T2DM (8.7%). Table 1 summarizes the characteristics of the study participants by sex. Men and women had similar ages, rates for diabetes or for taking antihyperglycemic drugs, plasma glucose, and total and LDL cholesterol levels. Men had a greater BMI (+1.0 kg/m^2) and waist circumference (+7.7 cm), higher systolic BP (+5.2 mm Hg), higher serum creatinine (+22.7 μmol/L), ALT (+10 U/L), and GGT (+18 U/L), higher serum triglycerides (+0.49 mmol/L), lower serum HDL cholesterol concentrations (−0.22 mmol/L), and higher proportions of current smoking (57.9% vs 1.5%) and alcohol intake (36.2% vs 1.9%). In addition, men, compared with women, had higher serum me-NAM concentrations (12.5 vs 11.7 ng/mL; $P = .002$).

Association of me-NAM with clinical parameters

We next investigated the relationship of serum me-NAM levels with various anthropometric and laboratory parameters. Serum me-NAM, triglyceride, ALT, and GGT levels were not normally distributed by the Shapiro-Wilks statistic (Supplemental Table 1). This can also been seen in the histogram and quantile-quantile plots for me-NAM (Supplemental Figure 1, A and B) and triglycerides, ALT, and GGT (not shown). After log transformation, me-NAM distribution was markedly improved and became virtually linear (Supplemental Figure 1, C and D). Thus, these parameters were logarithmically transformed for partial Pearson correlations. Serum me-NAM concentrations were positively associated with BMI and waist and hip circumference ($r = 0.09–0.22$; $P \leq .04$) and negatively with HDL cholesterol ($r = -0.09$ to -0.18; $P < .05$) in both men and in women (Table 2). In men, serum me-NAM levels were also positively associated with serum LDL cholesterol, triglycerides, fasting plasma glucose, ALT and GGT, and serum creatinine ($r = 0.10–0.11$; $P \leq .01$) (Table 2). All of these correlations remained statistically significant ($P \leq .02$) except for triglycerides and GGT after adjustments for age, current smoking, alcohol intake, BMI, systolic BP, serum ALT and GGT, creatinine, fasting plasma glucose, total and LDL cholesterols, and triglycerides, as appropriate (Table 2). Similar results were obtained for Spearman rho correlation analysis using non–log transformed clinical parameters (Supplemental Table 2).

Table 2. Simple and Multivariate Adjusted Correlations Between me-NAM and Clinical Parameters by Sex

	Serum me-NAM, ng/mL, log[a]							
	Men (n = 691)				Women (n = 469)			
	r	*P*	Partial *r*	*P*	*r*	*P*	Partial *r*	*P*
Age, y	−0.02	.62	0.04	.31	−0.01	.83	−0.02	.70
Systolic BP, mm Hg	−0.06	.10	−0.07	.08	0.02	.61	−0.01	.85
BMI, kg/m^2	0.22	<.001	0.15	<.001	0.13	.004	0.14	.002
Waist circumference, cm	0.22	<.001	0.14	<.001	0.09	.04	0.10	.03
Hip circumference, cm	0.22	<.001	0.15	<.001	0.12	.008	0.13	.007
Fasting plasma glucose, mmol/L	0.11	.003	0.11	.005	0.05	.24	0.05	.24
Total cholesterol, mmol/L	0.05	.15	0.03	.51	−0.02	.69	−0.007	.89
LDL cholesterol, mmol/L	0.11	.005	0.09	.02	0.04	.38	0.04	.05
HDL cholesterol, mmol/L	−0.18	<.001	−0.12	.002	−0.09	<.05	−0.13	.007
Triglycerides, mmol/L[a]	0.09	.02	−0.01	.72	−0.07	.12	−0.11	.02
ALT, U/L[a]	0.21	<.001	0.15	<.001	0.05	.27	0.02	.63
GGT, U/L[a]	0.10	.008	0.06	.10	0.02	.71	0.01	.86
Serum creatinine, μmol/L	0.10	.01	0.10	.006	0.03	.54	0.03	.56

[a] Log-transformed variable. First, we performed sex-specific simple correlation analyses to identify potential variables correlated with the serum me-NAM concentration. We further performed sex-specific multivariate adjusted correlation analyses of the relationship between ne-NAM and clinical parameters, while controlling for the above-identified correlates, as appropriate.

Association of me-NAM with obesity and diabetes

We next performed multiple logistic regression analysis to investigate whether me-NAM levels were associated with the risks for obesity and T2DM. Because men and women had different BMI and waist circumference, but similar diabetes prevalence (Table 1), we performed logistic regression analysis for obesity and T2DM in all subjects as well as in men and women separately. After adjustment for potential confounders (ie, age, sex, current smoking and alcohol intake, serum ALT and GGT, creatinine, fasting plasma glucose, total and LDL cholesterols, triglycerides, and systolic BP), elevated serum me-NAM concentrations were significantly associated with higher risks for overweight/obesity in all subjects (OR, 3.04; 95% CI, 1.61–5.73; $P < .001$), men (OR, 2.36; 95% CI, 1.10–5.08; $P = .03$), and women (OR, 5.78; 95% CI, 1.78–18.76; $P = .004$), respectively (Table 3). Similarly, me-NAM was significantly associated with higher risk for T2DM in all subjects (OR, 1.53; 95% CI, 1.14–2.05; $P = .005$), men (OR, 1.56; 95% CI, 1.10–2.22; $P = .01$), and women (OR, 1.86; 95% CI, 1.05–3.31; $P = .03$), respectively (Table 3). Similar trend and significance were obtained when we performed logistic regression using nontransformed me-NAM, although the OR of obesity was weaker (Supplemental Table 3).

Discussion

We found that serum me-NAM, an indicator of NNMT activity, is strongly associated with obesity and T2DM at a large-scale population level. Among 1160 Chinese adults in our study, high serum me-NAM concentrations are associated with an approximate 2-fold increase in the risk for obesity and a 53% higher risk for T2DM. Another interesting finding of the present study is that me-NAM negatively correlates with HDL, independent of body weight, diabetes, and other risk factors.

Until now, there have been 3 small studies that investigated me-NAM levels in obesity and T2DM. The first untargeted metabolomic analyses revealed high urinary me-NAM in humans with T2DM (11). The second study showed that me-NAM levels are higher in individuals with T2DM than in healthy control subjects 5 hours after a

Table 3. Associations of Serum me-NAM Concentrations With Obesity and Diabetes

	Serum me-NAM (+1 ng/mL), log					
	All (n = 1160)		Men (n = 691)		Women (n = 469)	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Overweight/obesity	3.04 (1.61–5.73)	<.001	2.36 (1.10–5.08)	.03	5.78 (1.78–18.76)	.004
Diabetes	1.53 (1.14–2.05)	.005	1.56 (1.10–2.22)	.01	1.86 (1.05–3.31)	.03

In a multiple logistic regression model, ORs (95% CIs) were adjusted for age, sex (not in men and women), BMI (not for overweight/obesity), systolic BP, current smoking and alcohol intake, fasting plasma glucose (not for diabetes), serum ALT and GGT, creatinine, total and LDL cholesterol, and triglycerides.

100-mg nicotinamide challenge (15). However, this smaller (n = 28) study did not find a statistical difference in me-NAM levels between T2DM and control subjects before the nicotinamide challenge, and BMI of the subjects was not reported. More recently, Kannt et al (9) measured me-NAM levels in 111 subjects without T2DM and 88 subjects with T2DM and demonstrated that me-NAM levels significantly correlated with the degree of insulin resistance in type 2 diabetes. The study did not report whether me-NAM was correlated with BMI in all subjects studied. Adipose NNMT mRNA levels, which correlate with serum me-NAM only in subjects with a BMI of >39 kg/m^2, are not associated with BMI. In our study, however, we observed that me-NAM strongly associated with BMI. This finding is consistent with the biological roles of NNMT in regulating energy expenditure in mouse studies (6). The discrepancies in association of me-NAM with obesity between our study and that of Kannt et al (9) may be due to several important factors including study design, sample size, obesity severity, and ethnicity. First, our study was population-based with 1160 subjects, which provided the possibility of finding context-dependent associations. Second, the mean BMI was 33 kg/m^2 in control subjects and 49 kg/m^2 in diabetic subjects in the cohort of Kannt et al. On the other hand, the mean BMI in our study was 23 to 24 kg/m^2. Finally, the prevalence of abdominal obesity is higher (2, 16), whereas nonalcoholic fatty liver disease is lower in Chinese (12.5%–24.5%) (17–19) than in Caucasians (33%–44%) (20, 21). It is possible that me-NAM levels in our cohort are less affected by hepatic NNMT activity. Further studies with more detailed clinical parameters, especially body composition and fatty liver index, will be helpful to clarify the associations of NNMT activity with adiposity and severity of fatty liver.

Another interesting observation of the current study is a negative correlation between me-NAM and HDL cholesterol, independent of other risk factors, in the general population. It has long been known that elevated homocysteine, another product of NNMT activity, is associated with low HDL (22) and cardiovascular disease (23). It is thought that elevated homocysteine may contribute to the development of cardiovascular disease. However, homocysteine lowering does not prevent cardiovascular disease or reduce all-cause mortality in patients with vascular disease or in primary prevention (24). Now our data show that me-NAM is also associated with low HDL. It is possible that increased NNMT activity, rather than elevated homocysteine levels, may contribute to low HDL and cardiovascular risk. In fact, hepatic NNMT activity increases by >2-fold in apolipoprotein-E/LDL receptor double knockout mice compared with that in control mice (25). Further studies are necessary to determine whether inhibition of NNMT activity may increase HDL levels and improve cardiovascular disease.

The mechanisms for increased NNMT activity and me-NAM levels in obesity and T2DM are unclear. NNMT is activated by its substrate, nicotinamide (26). Nicotinamide overload induces higher plasma me-NAM levels in individuals with T2DM than in healthy control subjects (15). In addition, treating mice with high-dose nicotinic acid activates NNMT in adipose tissue (8). Interestingly, there is an association between the timing of mandatory dietary nicotinamide (vitamin B_3) supplements and the timing of increased prevalence of obesity and T2DM (15). However, nicotinamide levels did not correlate with BMI, glucose, and lipid levels in our study (data not shown). This, however, does not rule out a potential role of nicotinamide in NNMT activation in obesity and T2DM because nicotinamide, as a precursor of NAD^+, is dynamically changed with NAD^+ synthesis and catabolism (27). NNMT expression and activity, especially in adipose tissue, appear to be regulated by nutritional status such as a high-fat diet. Further studies on the associations between nutritional factors such as trans fat (28, 29) and sugar-sweetened beverage (30) intake with NNMT activity will provide significant insights into the mechanisms for the regulation of NNMT activity. Nutrients can reverse or change epigenetic phenomena such as histone modifications, thereby modifying the expression of critical genes associated with energy expenditure. Because NNMT regulates the availability of the methyl group and affects histone methylation and downstream gene expression (6), NNMT may be a key mechanism linking nutrients, epigenetic regulation, and energy metabolism in the development of obesity.

Our study should be interpreted within the context of its limitations. The cross-sectional design does not allow causal inference. In addition, the association of serum me-NAM with obesity and diabetes was not a prespecified endpoint for the subjects recruited in the study.

In summary, our first large-scale population study shows significant associations of serum me-NAM with obesity and diabetes in Chinese. In addition, me-NAM negatively correlates with HDL. Although prospective and interventional studies are necessary to investigate whether elevated NNMT activity may play a causal role in obesity and T2DM in humans, our findings provide novel insights into the potential roles of NNMT in obesity, diabetes, lipid disorders, and cardiovascular disease.

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