

Research Paper

Excess nicotinamide increases plasma serotonin and histamine levels

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Abstract: Methylation, a methyl group-consuming reaction, plays a key role in the degradation (i.e., inactivation) of monoamine neurotransmitters, including catecholamines, serotonin and histamine. Without labile methyl groups, the methylation-mediated degradation cannot take place. Although high niacin (nicotinic acid and nicotinamide) intake, which is very common nowadays, is known to deplete the body's methyl-group pool, its effect on monoamine-neurotransmitter degradation is not well understood. The aim of this article was to investigate the effect of excess nicotinamide on the levels of plasma serotonin and histamine in healthy subjects. Urine and venous blood samples were collected from nine healthy male volunteers before and after oral loading with 100 mg nicotinamide. Plasma *N*¹-methylnicotinamide, urinary *N*¹-methyl-2-pyridone-5-carboxamide (2-Py), and plasma betaine levels were measured by using high-performance liquid chromatography (HPLC). Plasma concentrations of choline, serotonin and histamine were measured using commercial kits. The results showed that the plasma *N*¹-methylnicotinamide level and the urinary excretion of 2-Py significantly increased after oral loading with 100 mg nicotinamide, which was accompanied with a decrease in the methyl-group donor betaine. Compared with those before nicotinamide load, five-hour postload plasma serotonin and histamine levels significantly increased. These results suggest that excess nicotinamide can disturb monoamine-neurotransmitter metabolism. These findings may be of significance in understanding the etiology of monoamine-related mental diseases, such as schizophrenia and autism (a neurodevelopmental disorder).

Key words: nicotinamide; serotonin; histamine; monoamine neurotransmitters; mental disorders; autism

烟酰胺超载增加血中5-羟色胺和组胺水平

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摘要: 甲基化是单胺类神经递质儿茶酚胺、5-羟色胺和组胺降解的关键步骤。没有活泼甲基供应, 即使催化甲基化反应的转甲基酶正常, 甲基化介导的单胺类神经递质降解将不能进行。已知尼亚新(烟酸和烟酰胺)降解消耗体内活泼甲基, 而目前食物中添加尼亚新已使高尼亚新饮食非常普遍。然而, 过多尼亚新对单胺类神经递质代谢的影响尚不完全清楚。本文旨在观察烟酰胺超载对人5-羟色胺和组胺代谢的影响。9名健康男性志愿者禁食过夜后, 口服100 mg烟酰胺, 于口服药物前后取尿液和血浆样本。用高效液相色谱法检测血浆甲基化烟酰胺、甜菜碱和尿中*N*¹-甲基-2-吡啶酮-5-羟基胺水平, 用试剂盒检测血浆胆碱、组胺和5-羟色胺水平。结果显示, 烟酰胺负荷后, 受试者血浆甲基化烟酰胺水平和尿中甲基化代谢产物*N*¹-甲

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基-2-吡啶酮-5-羟基胺排出均增加，而血浆甲基供体甜菜碱水平降低，同时伴5-羟色胺和组胺水平显著增加。以上结果提示过量摄入烟酰胺可引起单胺类神经递质代谢紊乱，这将有助于揭示饮食与单胺代谢紊乱相关疾病(如精神分裂症和自闭症)的关系。

关键词：烟酰胺；5-羟色胺；组胺；单胺神经递质；精神疾病；自闭症

中图分类号：R338; R749

Monoamine neurotransmitters include catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, and histamine. Neuropsychiatric disorders, such as autism^[1] and schizophrenia^[2], exhibit an abnormal metabolism of monoamine neurotransmitters. Given that dietary factors play an important role in neuropsychiatric disorders^[3-5], there is the possibility that the mechanism underlying the effect of dietary factors may be due to an alteration in monoamine metabolism.

In order to obtain appropriate neurological and immune responses, released monoamines from nervous

system and immune system in response to stimuli must be timely removed through enzymatic degradation or reuptake. Methylation is one of the key steps in the degradation (i.e., inactivation) of monoamine neurotransmitters, which is an enzymatic, methyl-group consuming reaction (Fig. 1). Evidently, without labile methyl-group supply, methylation-mediated monoamine neurotransmitter degradation cannot take place.

Excess niacin (nicotinamide and nicotinic acid) mainly undergoes methylation-mediated degradation, and thus may deplete the methyl-group pool of the

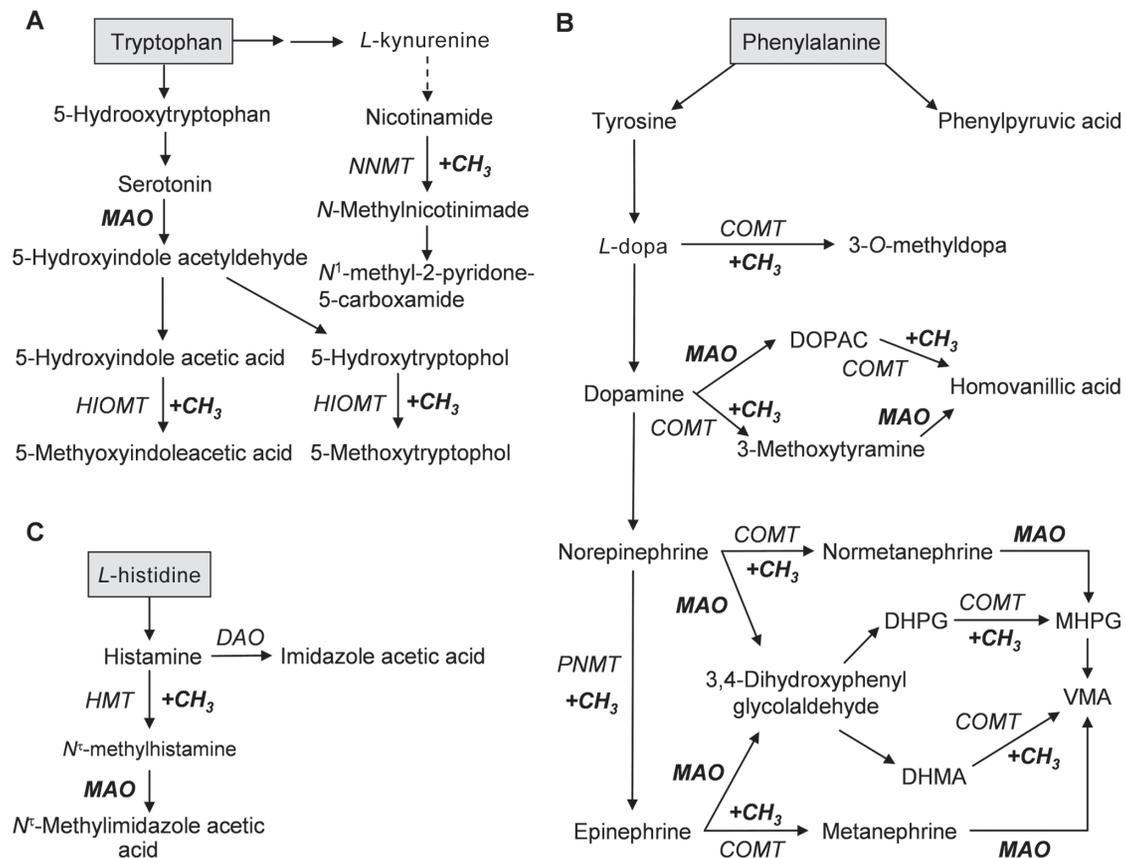


Fig. 1. The metabolism of monoamine neurotransmitters. A–C: The biosynthetic and degradative pathways of serotonin (A), catecholamines (dopamine, norepinephrine and epinephrine, B), and histamine (C). COMT, catechol-O-methyltransferase; DAO, diamine oxidase; DHMA, 3,4-dihydroxymandelic acid; DHPG, 3,4-dihydroxyphenyl glycol; DOPAC, 3,4-dihydroxyphenyl acetic acid; HIOMT, hydroxyindole-O-methyltransferase; HMT, histamine N-methyltransferase; MAO, monoamine oxidase; MHPG, hydroxyphenyl glycol; NNMT, nicotinamide N-methyltransferase; VMA, vanillylmandelic acid.

body^[6]. Thus, it is expected that niacin may affect the methylation-mediated degradation of monoamine neurotransmitters by competing for labile methyl groups. In our previous study we found that nicotinamide load increases the plasma norepinephrine, but decreases its methylated metabolites^[7]. In the present study, we further investigate the effect of excess nicotinamide on the plasma levels of serotonin and histamine in healthy subjects.

1 MATERIALS AND METHODS

1.1 Nicotinamide load test in humans

The present study was approved by the relevant ethics committee, and all the participants gave informed consent. Nine healthy male volunteers aged 41 to 58 years (mean, 50.8 years) participated in this study. All subjects refrained from drugs, alcohol and caffeinated products for at least 12 h before the study. After an overnight fasting, urine was collected and quantitated 1 h before, and 1, 2, 3, 4 and 5 h after oral loading with 100 mg nicotinamide (Lisheng Pharma, Tianjin, China). Venous blood was collected into sodium citrate tubes before and 5 h after nicotinamide loading, and separated by centrifugation (1 500 g, 10 min). Aliquots of each plasma and urine sample were placed directly in liquid nitrogen and then transferred to -80°C and -20°C , respectively.

1.2 Determination of nicotinamide metabolites and betaine

Plasma *N*¹-methylnicotinamide, urinary *N*¹-methyl-2-pyridone-5-carboxamide (2-Py), and plasma betaine levels were measured by using high-performance liquid chromatography (HPLC), as described previously^[7,8].

1.3 Assays of choline, serotonin and histamine

Plasma concentrations of choline, serotonin and histamine were measured using a Choline/Acetylcholine Quantification kit (Biovision, Mountain View, CA, USA), a Histamine ELISA kit (IBL, Hamburg, Germany), and a Serotonin ELISA kit (IBL, Hamburg, Germany), respectively. The absorbance of each plate was measured by a Bio-Rad model 550-microplate reader (Bio-Rad Co., Hercules, CA, USA).

1.4 Statistical analysis

The data are presented as means \pm SEM. Statistical differences in the data were evaluated by paired Student's *t* test using SPSS software (SPSS Inc., Chicago, USA), and were considered significant at $P < 0.05$.

2 RESULTS

2.1 Effect of nicotinamide loading on plasma *N*¹-methylnicotinamide and urinary 2-Py levels

As shown in Fig. 2A, nicotinamide load induced a significant increase in the urinary excretion of 2-Py (the major methylated metabolite of nicotinamide). The 5-hour postload urinary excretion of 2-Py was still significantly higher than that before nicotinamide load ($4.50 \text{ mg} \pm 0.34 \text{ mg}$ vs $0.92 \text{ mg} \pm 0.12 \text{ mg}$). These results indicate that methylation-mediated nicotinamide degradation occurs very rapidly in the body. The 5-hour

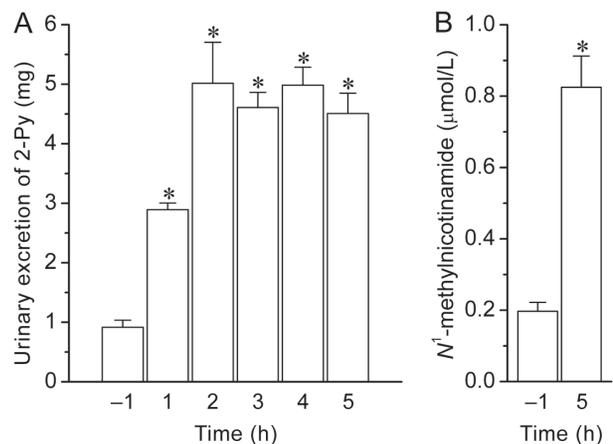


Fig. 2. Nicotinamide load-induced changes in plasma *N*¹-methylnicotinamide level and urinary *N*¹-methyl-2-pyridone-5-carboxamide (2-Py) excretion. A: Urinary excretion of 2-Py at different times. B: Plasma *N*¹-methylnicotinamide levels at 1 h before and 5 h after nicotinamide load. Means \pm SEM, $n = 9$. * $P < 0.001$ vs before nicotinamide load (-1 h).

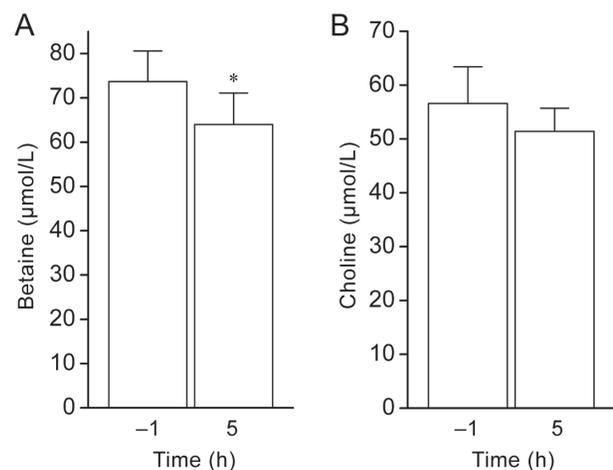


Fig. 3. Plasma betaine (A) and choline (B) levels before and 5 h after nicotinamide load. Means \pm SEM, $n = 9$. * $P < 0.05$ vs before nicotinamide load (-1 h).

postload plasma *N*¹-methylnicotinamide was significantly higher than the baseline value (Fig. 2B).

2.2 Nicotinamide loading decreased plasma betaine level

Accompanied with the increased urinary excretion of 2-Py, there was a significant decrease in the plasma level of betaine 5 h after nicotinamide load (Fig. 3A). The plasma choline concentration also showed a decrease trend, but there was no statistically significant difference compared with baseline value ($P > 0.05$, Fig. 3B). These results indicate that excess nicotinamide decreases the methyl-group pool size.

2.3 Nicotinamide loading increased plasma serotonin and histamine levels

We then examined the effect of nicotinamide load on plasma serotonin and histamine levels. The result showed that the 5-hour postload plasma serotonin level was significantly higher than the value before nicotinamide load ($P < 0.05$, Fig. 4A). Similarly, the level of plasma histamine was also significantly increased (Fig. 4B). This study demonstrated that excess nicotinamide may play a causal role in increased plasma serotonin and histamine levels.

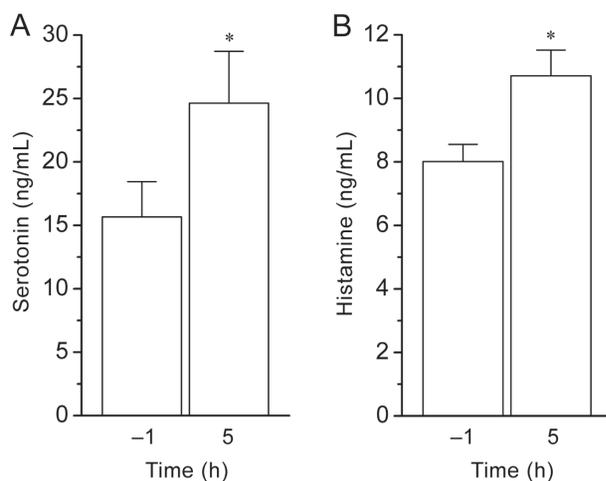


Fig. 4. Plasma serotonin (A) and histamine (B) levels before and 5 h after nicotinamide load. Means \pm SEM, $n = 9$. * $P < 0.05$ vs before nicotinamide load (-1 h).

3 DISCUSSION

This study found that nicotinamide load reduced the methyl-group pool associated with increases in plasma serotonin and histamine levels. The present findings, together with earlier results showing that nicotinamide load increased plasma norepinephrine but decreased its

methylated derivatives^[7], suggest that excessive nicotinamide could inhibit the degradation of monoamine neurotransmitters presumably due to methyl-group pool depletion.

In humans, nicotinamide is degraded mainly via methylation to produce methylated metabolites, *N*¹-methyl nicotinamide and 2-Py^[6]. Evidently, excess nicotinamide can increase the consumption of labile methyl groups. Betaine serves as a methyl donor in a reaction converting homocysteine to methionine, whereas choline can be converted to betaine in the liver and kidney^[9]. Therefore, the levels of plasma choline and betaine are indicators of the size of methyl-group pool of the body. The present findings that nicotinamide load had a more profound influence on plasma betaine than choline suggest that betaine is a more effective methyl donor than choline.

Niacin is usually classified as a B vitamin. However, strictly speaking, it is not a vitamin because it can be synthesized from tryptophan. As shown in Fig. 1, tryptophan is degraded through tryptophan-kynurenine-niacin and tryptophan-serotonin pathways. These two pathways function together to regulate tryptophan homeostasis. For example, an increase in nicotinamide intake can lead to an increase in urinary excretion of 5-hydroxyindoleacetic acid, a metabolite of serotonin^[10], suggesting an increase in tryptophan degradation through tryptophan-serotonin pathway (i.e., an increase in serotonin synthesis). Moreover, nicotinamide may affect methylation-mediated serotonin degradation by competing for methyl groups. Therefore, high nicotinamide intake may increase serotonin levels by a mechanism of increased synthesis and decreased degradation.

Serotonin and histamine act not only as neurotransmitters, but also function as important signaling molecules in the skin, gastrointestinal tract and immune system^[11,12] on the one hand, and on the other hand, neuropsychiatric disorders, such as autism^[13] and schizophrenia^[14], are often associated with immune abnormalities, besides disturbed monoamine-neurotransmitter metabolism^[1,2]. The finding that nicotinamide load increases the plasma levels of both serotonin and histamine, suggests that excess nicotinamide load could affect both the nervous system and the immune system. Although not proved, high nicotinamide intake, which is very common nowadays due to mandatory vitamin fortification^[15], may play a role in the association between abnormal metabolism of monoamine neurotransmitters and immune abnormalities in neuropsychiatric disorders.

Indeed, ecological evidence has revealed an association between the prevalence of schizophrenia and a higher national dietary intake of refined sugar and dairy products^[3], which are vehicles for vitamin fortification^[15].

As shown in Fig. 1, methylation catalyzed by distinct methyltransferases and oxidation catalyzed by the monoamine oxidase (MAO) are two necessary steps in the degradation of monoamine neurotransmitters. If methylation is interrupted due to methyl-group depletion, the function of MAO will become increasingly crucial to monoamine-neurotransmitter degradation. The isoenzymes of MAO, MAO-A and MAO-B, are X-linked enzymes^[16], and the activity of MAO in women is stronger than that of men^[17]. Thus, under the same methyl-group-pool depletion condition, men should be more prone to mental disorders than women. Indeed, a body of evidence has been accumulated, suggesting that abnormal MAO-A activity is implicated in several neuropsychiatric disorders, such as depression, autism, and attention deficit hyperactivity disorder, which show sexual dimorphism^[18-21].

The results of this study may be of significance to understand the etiology of mental disorders, such as autism which is a pervasive developmental disorder manifested in the first 3 years of life by dysfunction in social interaction and communication^[22]. The most consistently observed biological findings in autism are elevated serotonin levels in the blood and immunological abnormalities^[13]. Since autism was first described by Kanner in 1943^[23], its prevalence has dramatically increased from 4 to 5 per 10 000 children in 1940s to approximately 1 in 88 in the United States today^[22]. Notably, the increasing prevalence of autism is accompanied with a significant increase in the per capita consumption of niacin in the United States due to the implementation of mandatory niacin fortification (i.e., addition of synthetic nicotinamide to grain products)^[15]. Especially, infant formulas and children's foods contain much higher amounts of nicotinamide, for example, the niacin (in the form of nicotinamide) contents of most infant formulas in U.S. market range from 1 000 to 1 950 µg/100 kcal, which is more than five times that of human milk (ranging 164–343 µg/100 kcal^[24]). Thus, formula-feeding will increase the risk of nicotinamide overload. This notion is supported by the observation of high urinary excretion of niacin metabolites in autistic subjects^[25]. Thus, the present findings that nicotinamide load increases the levels of plasma serotonin and histamine imply that high nicotinamide exposure

may be a risk factor for autism, but further studies are needed to confirm this, especially in autistic children.

In summary, excess nicotinamide increases the plasma monoamine-neurotransmitter levels. The present findings provide evidence for the potential role of nicotinamide in the metabolic disorder of monoamine neurotransmitters.

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