

## 学 位 論 文 の 要 旨

論文題目 Nutritional study on the influence of dietary vitamin B<sub>6</sub> on colon luminal environment and heart (食餌ビタミンB<sub>6</sub>の腸内環境、及び心臓に及ぼす影響に関する栄養学的研究)

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### Introduction

Vitamin B<sub>6</sub> (B<sub>6</sub>) is an essential water-soluble vitamin required for normal growth and development in mammals. B<sub>6</sub> is the collective term for metabolically and functionally compounds including pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and their phosphorylated forms pyridoxal 5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP), and pyridoxamine 5'-phosphate (PMP). PLP is the biologically active form of B<sub>6</sub>, acts as a co-factor in over 140 distinct enzyme reactions that are involved in the metabolisms of proteins, lipids, and carbohydrates, neurotransmitters, nucleic acids, one carbon units, and immune modulatory metabolites and others. After intestinal absorption, most of B<sub>6</sub> ingested is transported to the liver and taken up by facilitated diffusion. After being phosphorylated by pyridoxal kinase, PNP and PMP are converted to PLP.

Beyond its the role as the co-factor, B<sub>6</sub> has the preventive roles in certain diseases including brain diseases such as Parkinson's disease (PD), Schizophrenia, Alzheimer disease (AD) and autoimmune diseases such as rheumatic arthritis (RA). B<sub>6</sub> also have important role in heart diseases such as cardiovascular disease (CVD), atherosclerosis, and stroke and colon diseases such as irritable bowel disease (IBS), intestinal bowel diseases (IBD), colitis, and colon cancer (CRC). However, the underlying mechanisms of these preventive effects of B<sub>6</sub> are still unclear. In this study, I focused on the preventive effects of B<sub>6</sub> on colon and heart diseases.

### Effect of dietary supplemental vitamin B<sub>6</sub> and gender difference on colon luminal environment

Accumulating studies have suggested the preventive role of dietary B<sub>6</sub> on colon diseases. B<sub>6</sub> has the potential mechanisms to prevent from colon diseases include reducing cell proliferation, oxidative stress, inflammation, angiogenesis, and increased fecal mucins. It has been reported that The status of B<sub>6</sub> is affected by gender difference. There is growing evidence that the incidence of colon diseases is affected by gender difference. However, the underlying mechanisms of the effects of gender on colon diseases are still unclear.

An understanding of the colon luminal environment is essential to reveal the effects of dietary B<sub>6</sub> and gender difference on colon diseases. The important factors in the luminal environment includes intestinal mucins, IgA, microflora, etc. In this study, I hypothesized that gender difference modulates the colon luminal environment, which is dependent upon B<sub>6</sub> status. To investigate this hypothesis, male and female rats were fed the diet containing 1 mg (low), 7 mg (recommended), or 35 mg (high)

pyridoxine HCl/kg diet for 6 weeks.

As a result, dietary B<sub>6</sub> significantly increased fecal mucins and the effect was particularly profound in the female rats. The fecal mucin levels were significantly correlated with colon free threonine and serine and with gene expression of colon MUC16, implying that the combined effect of gender and dietary B<sub>6</sub> on fecal mucins was mediated by the alteration in the levels of such amino acids and MUC16 expression. Meanwhile, this study indicated no effect of gender and B<sub>6</sub> on fecal IgA. Mucins are considered to play a beneficial role in intestinal barrier function and in preventing colon cancer. Thus, dietary B<sub>6</sub> and gender may modulate colon luminal environment through mucin production, but not IgA production. The abundances of cecal and fecal *Akkermansia muciniphila* (mucin degrader) were unaffected. Thus, *Akkermansia muciniphila* is not likely to be responsible for the alteration in mucins. This study further showed the significant effects of gender difference on colon free amino acids such as threonine, ornithine, asparagine/aspartate ratio, glutamine/glutamate ratio, on cecal and fecal *Lactobacillus* spp. abundance and colon gene expressions of MUC16 and TLR8, the factors relating to colon health and diseases. Accordingly, my study suggests that dietary B<sub>6</sub> and gender difference may have an impact on colon diseases by modulating these parameters.

#### **Effect of dietary supplemental vitamin B6 on the levels of anti-disease metabolites in heart**

Several epidemiological studies have shown the positive benefits of B<sub>6</sub> to heart diseases. The suggested mechanisms responsible for the preventive effects of B<sub>6</sub> against heart diseases include homocysteine, purinergic receptors signaling, inflammation, and kynurenine pathway. However, the exact mechanisms of the effects of B<sub>6</sub> are still unclear.

In this study, I hypothesized that dietary B<sub>6</sub> improves heart dysfunction by modulating amino acid metabolisms. To examine this hypothesis, I conducted metabolomics analysis to investigate concentrations of metabolites of the heart in rats fed a diet containing 1 mg (low) or 35 mg (high) pyridoxine (PN) HCl/kg for six weeks.

As a result, there were over 500 metabolites detected, and 21 metabolites were affected by dietary B<sub>6</sub>. The first group affected by supplemental B<sub>6</sub> was the metabolites related to carnosine were carnosine, anserine, homocarnosine, and  $\beta$ -alanine. The second group was the amino acids such as alanine, serine, isoleucine, leucine, valine, methionine. The third group was the metabolite in the TCA cycle and urea cycles such as malic acid, fumaric acid, argininosuccinic acid, and ornithine. The fourth group is the other metabolites such as gamma-aminobutyric acid (GABA), histamine, 1H-Imidazole-4-propionic acid,  $\gamma$ -butyrobetaine, carnitine, adenine, and FAD. These metabolites except for ornithine were significantly increased by high B<sub>6</sub> diet, whereas ornithine was increased by the low B<sub>6</sub> diet. Among those metabolites, carnosine, anserine,  $\beta$ -alanine, GABA, histamine, fumaric acid, malic acid, carnitine, and adenine are known to have heart-protective effects.

Carnosine is synthesized from histidine and  $\beta$ -alanine by carnosine synthase. Meanwhile anserine is produced from carnosine by carnosine-N-methyltransferase. In this study, high B<sub>6</sub> diet markedly elevated the concentration of carnosine, anserine and their precursor,  $\beta$ -alanine. There was strong correlation of  $\beta$ -alanine with carnosine and anserine levels. Thus, increased carnosine and anserine by dietary B<sub>6</sub> might be, at least in part, caused by increased  $\beta$ -alanine.

availability of GABA affects homocarnosine synthesis. In this study, homocarnosine levels had a

strong correlation with GABA levels. Thus, increased homocarnosine by dietary B<sub>6</sub> might relate to higher GABA.

Histamine is derived from the decarboxylation of histidine, a reaction is catalyzed by the enzyme histidine decarboxylase, a PLP-dependent enzyme. Thus, the higher histamine levels by high B<sub>6</sub> diet might be mediated by higher conversion from histidine.

The concentration of  $\beta$ -alanine and GABA were increased by dietary B<sub>6</sub> and inversely associated with ornithine concentration. Ornithine is converted by ornithine decarboxylase to  $\beta$ -alanine in polyamines pathway. Meanwhile GABA is synthesized by aminotransferase from ornithine through glutamic acid pathways. Thus, high B<sub>6</sub> diet might increase the formation of  $\beta$ -alanine and GABA from ornithine through the two pathways.

Further HPLC analysis showed that the recommended and high levels of B<sub>6</sub> in the diet-markedly elevated the concentration of GABA and histamine compared to low B<sub>6</sub> diet. Meanwhile, there was no significant difference in the concentrations of GABA and histamine between the recommended and high levels of B<sub>6</sub> diet. These results imply that the elevations in GABA and histamine can be produced by the supplementation of B<sub>6</sub> to the low B<sub>6</sub> diet, but not by the supplementation to normal diet of recommended B<sub>6</sub> level.

$\gamma$ -Butyrobetaine is a precursor for carnitine. Therefore, the higher concentration of  $\gamma$ -butyrobetaine by high B<sub>6</sub> diet might be ascribed to the increased concentration of carnitine.

The underlying mechanisms of the alterations of other metabolites such as alanine, serine, isoleucine, leucine, valine, methionine, fumaric acid, malic acid, argininosuccinic acid, butyrobetaine, carnitine, adenine and FAD by dietary B<sub>6</sub> are still unclear at present.

The results suggest that the possible mechanism of the heart-protective effect of B<sub>6</sub> is ascribed to increased heart-protective metabolites. My study explains the reason why adequate B<sub>6</sub> status is essential to maintain the optimal heart health.

## **Conclusion**

These study demonstrated supplemental B<sub>6</sub> increased fecal mucins in rats in a gender-dependent manner. The mechanisms of the effect of dietary B<sub>6</sub>, combined with gender difference, may be mediated by modulating colon free threonine and serine and gene expression of MUC16. During this study, I found the gender difference modulated several parameters, including colon free amino acids, mucins, microflora, and expressions of MUC16 and TLR8, important to colon diseases. The results imply that the effects of gender on colon diseases are mediated through alterations in such parameters. Further, I found the increased levels of several heart-protective metabolites such as carnosine, anserine, GABA, histamine, etc. in the heart of rats by supplemental B<sub>6</sub>. The possible mechanisms of increases in carnosine, anserine, GABA and histamine by supplemental B<sub>6</sub> were discussed. Collectively, the results imply the novel mechanisms of anti-heart disease effect of dietary B<sub>6</sub> by elevating such protective metabolites. Taken together, my Ph.D. studies give an insight into the understanding of the possible mechanisms of the beneficial effects of dietary B<sub>6</sub> on colon and heart.