

学位論文の要旨

Study on the influence of dietary vitamin B6 on heart and skeletal muscles

(食餌ビタミン B6 の心臓, 及び骨格筋に及ぼす影響に関する研究)

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Increasing evidence indicates that lower status of vitamin B6 is related to higher risk of coronary heart disease and atherosclerosis. Vitamin B6 is also reported to enhance the endurance of skeletal muscles. Furthermore, a case-study demonstrated that vitamin B6 supplementation is beneficial for McArdle's disease, a glycogenetic myopathy.

Pyridoxal-5'-phosphate (PLP), the active form of vitamin B6, acts as a co-factor for several enzymes involved in amino acid metabolism. Some reports indicate vitamin B6 deficiency modulates free amino acids concentrations in blood and tissues. On the other hand, certain amino acids and related metabolites including the branched-chain amino acids, carnosine, and β -alanine were reported to enhance muscle performance. Furthermore, functional amino acids, including arginine, glutamine, glutamate, leucine, and proline, are very promising for the prevention and treatment of metabolic diseases such as obesity, diabetes, and cardiovascular disorders. Nonetheless, there is limited information that links dietary vitamin B6 intake with amino acid metabolism in the heart and skeletal muscles.

Recent evidence suggests marginal vitamin B6 deficiency is common in both USA and Japan. Accordingly, this study investigated the effect of dietary vitamin B6 supplementation compared to a marginal vitamin B6-deficient diet on the concentrations of free amino acids and

related metabolites in heart and skeletal muscles, and on the gene expression in skeletal muscle of rats.

Effect of dietary vitamin B6 on the level of histidine dipeptides in heart

In the first study, I focused on the effect of dietary vitamin B6 on the concentrations of amino acids and related metabolites in heart of rats. Male rats were fed a diet containing 1, 7, or 35 mg pyridoxine (PN) HCl/kg for 6 weeks. As a result, the concentrations of heart carnosine (β -alanyl-L-histidine) and anserine (β -alanyl-N-methyl-L-histidine) in the 7 and 35 mg PN HCl/kg (the recommended level and excessive dietary level of vitamin B6 without any toxic symptom, respectively) groups were markedly greater than in the 1 mg PN HCl/kg (marginal vitamin B6 deficient level) group. Carnosine is reported to exert anti-oxidant, anti-inflammatory, and anti-ischemic effects on the heart. In cardiac myocytes, carnosine is suggested to be a modulator of intracellular calcium and contractility. Anserine, methylated analogue of carnosine, also exerts anti-oxidant and anti-inflammatory effects. Thus, the present results imply that dietary supplemental vitamin B6, correcting marginal vitamin B6-deficient status, might be favorable for heart function by elevating these histidine dipeptides.

Moreover, dietary vitamin B6 supplementation significantly increased serum carnosine concentration. Physical exercise was recently suggested to release carnosine from the skeletal muscles into the bloodstream. The higher concentration of carnosine caused by exercise is considered to decrease blood pressure in rats and humans. On the other hand, vitamin B6 supplementation is reported to attenuate blood pressure in rat models of hypertension. Thus, the elevated serum carnosine concentration caused by dietary vitamin B6 supplementation may at least partially relate to the favorable effect of vitamin B6 on circulation.

Effect of dietary vitamin B6 on the level of histidine dipeptides in skeletal muscles

Carnosine and anserine are abundant dipeptides in skeletal muscle. They constitute an integral part of skeletal muscle contractility and homeostasis, presumably through their role as anti-oxidant, pH-buffering, anti-glycation and/or calcium regulator. I hypothesized that dietary vitamin B6 also plays an important role in maintaining carnosine and anserine in skeletal muscles. Accordingly, in the second study I investigated the effects of dietary vitamin B6 supplementation compared to a marginal vitamin B6-deficient diet on the levels of these metabolites in the skeletal muscles of rats. Male and female rats were fed a diet containing 1, 7, or 35 mg PN HCl/kg for 6 weeks. In the gastrocnemius muscle of male rats, carnosine concentration was markedly higher in the 7 and 35 mg PN HCl/kg groups than in the 1 mg PN HCl/kg group, whereas that in the soleus muscle of male rats was significantly higher only in the 7 mg PN HCl/kg group than in the 1 mg PN HCl/kg group. In both muscles of female rats, carnosine concentration was remarkably higher in the 7 and 35 mg PN HCl/kg groups than in the 1 mg PN HCl/kg group.

It is noteworthy that the concentration of β -alanine, a precursor of carnosine, in gastrocnemius muscles was markedly elevated by dietary supplemental vitamin B6. This result suggested that the elevation of carnosine by dietary supplemental vitamin B6 might be partly due to elevated β -alanine by dietary supplemental vitamin B6. Animal study suggested β -alanine as one of the metabolites resulted from the injection of spermidine or spermine, which are produced by PLP dependent ornithine decarboxylase from ornithine. Interestingly, my result indicated significant inverse correlation between ornithine and β -alanine concentrations in gastrocnemius muscle, which shows more remarkable response of β -alanine to dietary vitamin B6 compared to soleus muscle. This raises the possibility that the elevation in the level of β -alanine in the gastrocnemius muscle by dietary supplemental vitamin B6 is at least in part mediated by enhancing the conversion from ornithine to β -alanine in skeletal muscles.

In order to explore the relevance of my findings to humans, I additionally investigated the possible relationship between vitamin B6 status and muscle carnosine content in human volunteers. In humans, lower muscle carnosine content was found in soleus muscle of women of the lower plasma PLP tertile, but this was not observed in gastrocnemius muscle or in men. My second study concluded that adequate dietary vitamin B6 is essential for maintaining carnosine in skeletal muscles of rats. Thus, the finding in rats could only be partly confirmed in human volunteers.

Effect of dietary vitamin B6 on gene expression in skeletal muscle

In the second study, the results showed that carnosine and PLP concentrations were markedly elevated by dietary supplemental vitamin B6. Both blood carnosine and PLP concentrations are also reported to be elevated by exercise. Thus, in the third study, I hypothesized that dietary supplemental vitamin B6 has a significant impact on skeletal muscles. To examine this possibility, I investigated the effect of vitamin B6 on the expressions of genes of factors relating to exercise, such as myokines and others.

Myokines, muscle endocrine product, are released when muscle contraction is occurred, such as exercise. A study reported that exercise up-regulated both antioxidant enzymes and antioxidant defense system relating to Nrf2. Expression of myogenin is reported to be induced by acute exercise in human skeletal muscle. HSP60 is also increased in cardiac and skeletal muscle after endurance training. Male rats were fed a diet containing 1, 7, or 35 mg PN HCl/kg for 6 weeks. Gastrocnemius muscle was used for Real time PCR analysis. Compared to the marginal vitamin B6-deficient diet (1 mg PN HCl/kg), the recommended level of dietary vitamin B6 (7 mg PN HCl/kg) elevated the expressions of several myokines genes, Nrf2-regulated genes, myogenin, and HSP60. However, excessive dietary level of vitamin B6 (35 mg PN HCl/kg) was less effective.

This implies the recommended level of dietary vitamin B6 is critical for the expressions of these genes in gastrocnemius muscle of rats. Intriguingly, most of the expressions of such genes were well correlated each other. Of great interest is that the elevations in the expression of such genes by dietary supplemental vitamin B6 appear to be similar to those by exercise.

In conclusion, these studies indicated dietary vitamin B6 supplementation is a determinant of carnosine concentration in the heart, skeletal muscles, and serum of rats. To our knowledge, this is the first evidence indicating dietary intake of a nutrient close to the range of regular daily intake plays a pivotal role in maintaining carnosine concentration in heart and skeletal muscles. Some of the findings in skeletal muscle could only be partly confirmed in human volunteers. Carnosine has been considered to be beneficial for heart disease and to have ergogenic effects. My study further indicated dietary vitamin B6 is a determinant of gene expressions of several factors playing important roles in skeletal muscle. Taken together, my studies provide a novel insight into the role of vitamin B6 in the heart and skeletal muscles.