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THE EFFECTS OF DIETARY FOOD FORTIFIED WITH VITAMIN B4 ON LIPID PROFILES IN SERUM AND LIVER TISSUE

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Abstract

The effects of dietary food fortified with vitamin B4 on lipid profiles in serum and liver tissue were studied. Rats were paired-fed a 0.25% vitamin B4 diet or a diet without vitamin B4 for 10 days. Serum lipid levels were measured using enzyme assay kits. Lipids of liver tissues were extracted and the lipid contents were determined. A piece of liver was prepared to determine the activities of fatty acid synthase (FAS) and fatty acid β-oxidation. The results showed that animals fed a food fortified with vitamin B4 had higher level of serum TG, PL, total cholesterol, and high density lipoprotein. Their increases were approximately by 74%, 20%, 27%, and 27%, respectively. The significant changes in liver lipid were only found in PL component. This treatment promoted FAS activity, but impaired the fatty acid β-oxidation. In conclusion: Dietary food fortified with vitamin B4 induces hypertriglyceridemia and liver PL level.

Keywords: cholesterol, high density lipoprotein, serum lipid, triglyceride, vitamin B4

Introduction

It is natural that vitamin B4 or adenine is a nucleobase (a purine derivative) with a variety of roles in biochemistry including an integral part of the structure of many coenzymes such as nicotinamide adenine dinucleotide (NAD) and flavine adenine dinucleotide (FAD), and the adenosine diphosphate (ADP). The NAD, FAD, and ADP molecules play major roles in cellular respiration. Adenine is also parts of nucleic acids, deoxyribonucleotide acid (DNA) and ribonucleotide acid (RNA), in which both latter molecules play important roles in protein synthesis.[1] A modified form of adenosine monophosphate is thought to be a secondary messenger in the propagation of many hormonal stimuli. It was also reported that the extracellular adenosine plays as a signaling molecule to mediate divers' medical effects via cell surface receptor termed adenosine triphosphate (ATP) and ADP receptors.[2] Overall, vitamin B4 is one of the key molecules of the non-protein nitrogen compound found in all living cells.

Both DNA and RNA nucleotides are synthesized from amino acid by de novo or from the waste of nucleotide by salvage pathway. Unlike de novo that is high cost pathway, the nucleotides resulting from salvage pathway are much simpler.[3] Because nucleotides are the structural units of the nucleic acids and essential compounds in energy transfer systems (in ATP form), they have been assumed to play an important role in lipid metabolisms.[4] Lipid metabolism plays an important role in regulation of life- style related diseases such as hypertension, diabetes mellitus, atherosclerosis, etc. Those life-style related diseases are normally detected by the profiles of serum and/or plasma of bloodstream. Hence, a serum lipid profile generally indicates how lipid metabolism occurred in the liver. An exogenously applied vitamin B4 is taken up by cells and converted to nucleotides intracelullarly. Dietary food rich in vitamin B4 such as human and animal milks was helpful in cellular lipid metabolisms[5]. It was, however, not known yet that the serum and liver lipid differentiations were induced by the intake of vitamin B4. The present work was the first study to know the effect of dietary food fortified with vitamin B4 on lipid profiles in serum and liver tissue.

Methods

Animals and diets. All aspects of the experiment were conducted according to guidelines provided by the ethical committee of experimental animal care at Saga University (Saga, Japan). Male Sprague-Dawley (SD) rats aged 5 weeks were housed individually in an air-conditioned room (24°C) with a 12-h light/dark cycle. After a one-week adaptation period, rats were assigned

to two groups (five rats each). Control diet (as control group) was prepared according to recommendations of the American Institute of Nutrition (AIN) and contained (in weight %) 20 of casein, 10 of safflower oil, 1 of vitamin mixture (AIN-93), 3.5 of mineral mixture (AIN-93), 0.20 of choline bitartrate, 0.3 of DL-Methionine, 5 of cellulose, 15 of α-cornstarch, and sucrose to make 100. The vitamin B4 diet (as vitamin B4 group) was prepared by supplementation of 0.25% vitamin B4 to the control diet at the expense of sucrose. The animals received the diets for 10 d. At the end of the feeding period, rats were killed by decapitation after a 9-h starvation. Livers were excised immediately, and serum was separated from blood.

Analyses of serum and liver lipids. Liver lipids were extracted according to the method of Folch et al.[6] and concentrations of triglyceride (TG) and phospholipids (PL) were measured by the methods of Fletcher[7] and Bartlett,[8] respectively. The total cholesterol content was measured by the methods of Sperry and Webb with a minor adaptation.[9] Serum TG, PL, and cholesterol were measured using enzyme assay kits from Wako Pure Chemicals according to the manufacture's instructions.

Preparation of liver subcellular fractions. The mitochondrial and cytosol of liver sub cellular fractions were prepared as previously reported by Nagao et al.[10] Protein concentration was determined by the method of Lowry et al.[11]

Assays of hepatic enzyme activity. The lipogenic enzyme determined was fatty acid synthase (FAS; EC2.3.1.85). The enzyme activities of FAS were determined as previously described by Nagao et al.[10] The lipolytic enzyme determined was carnitine palmitoyl transferase-1 (CPT; EC2.3.1.23), a rate-limiting enzyme of fatty acid β-oxidation. The enzyme activities of CPT were also measured as previously reported by Nagao et al.[10]

Statistical analyses. All values are expressed as mean ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance, and all differences were inspected by Duncan's new multiple-range test using SSPS statistical software (SPSS inc., Chicago, IL, USA).[12] $p < 0.05$ was considered statistically significant.

Results and Discussion

Dietary food fortified with vitamin B4 reduced body weight and liver weight slightly. The daily food intake was paired-fed for each animal in order to get the same quantity of macronutrients, vitamins, and minerals ingested. The macronutrient as sources of caloric food of each group was prepared in excess amount. It was, therefore, the addition of food supplements, vitamin B4 compound, to the control diet provided the same quantity of the energy needed by each animal's homeostasis metabolisms of each group during the time course. Thus, the effect of intake vitamin B4 by the given concentration could be elucidated from this experimental design.

The daily adjusted food intake is shown in Table 1. Although food intakes were nearly similar between the groups, the final body weight and the liver weight of vitamin B4 group slightly decreased. Therefore, dietary food fortified with vitamin B4 might have body weight management properties and attenuates enlargement of liver size.

Dietary food fortified with vitamin B4 promoted serum triglycerides and HDL levels. As shown in Fig. 1, lipid levels in serum increased. Serum TG, PL, and total cholesterol of vitamin B4 group increased approximately by 74%, 20%, and 27%, respectively, than that of the control group. The high-density lipoprotein (HDL)-cholesterol also increased significantly ($p < 0.05$). The enhancement of serum TG level might indicate that this treatment induces secretion of very low density lipoprotein (VLDL) from liver into extrahepatic tissues.

Table 1. The Effects of Dietary Food Fortified with Vitamin B4 on Growth Parameters*

Group	Control diet	Vitamin B4
Initial body weight (g)	166 ± 4	165 ± 5
Final body weight (g)	210 ± 3	204 ± 4
Food intake (total, in gram)	154 ± 0	153 ± 2
Liver weight (g/100 g body weight)	4.4 ± 0.2	4.1 ± 0.1

* Rats were paired-fed vitamin B4 supplemented diet or a diet without vitamin B4 (control diet) for 10 days. Rats were killed by decapitation after a 9-h starvation. Values are expressed as mean± SEM of five rats. See Materials and Methods for composition of diets.

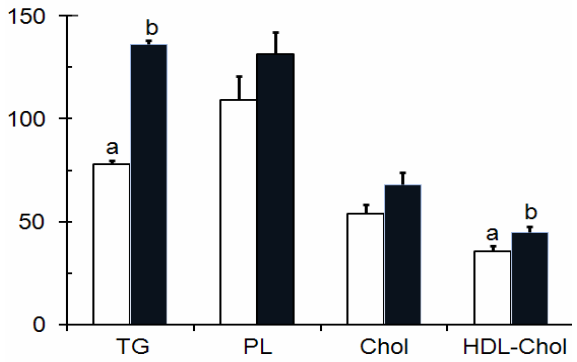

Figure 1. Serum Lipids Level (mg/dL), for Control Group (□) and Vitamin B4 Group (■)

Rats were paired-fed vitamin B4 supplemented diet or a diet without vitamin B4 (control diet) for 10 days. Rats were killed by decapitation after a 9-h starvation. Values are expressed as mean± SEM of five rats. Clearly define a & b regarding difference of significance at $p < 0.05$.

It was because VLDL is a lipoprotein handling the transportation of lipid compounds from the liver into extrahepatic tissues as well as reported previously that the increasing lipid packed into the VLDL particle results in a high concentration of lipid found in serum.[3] The promotion of serum lipid level found in the present study might indicate that dietary food fortified with vitamin B4 induces hyperlipidemia in rats.

Although serum total cholesterol slightly increased by this treatment (Fig. 1), it was accompanied with the increase of serum HDL level. The promotions of HDL level in serum blood that indicated the reverse flow of cholesterol from the extrahepatic into the liver was stimulated by the treatment. This mechanism might imply that the existing cholesterol overloads in the bloodstream undergo recycles in the liver and keep to the base line level in the bloodstream. This is in agreement with the reports of Elliot et al.[14] that the overload of cholesterol in extrahepatic cell membranes is transferred to HDL and thereafter the HDLs transfer their cholesterol esters into chylomicrons (when present), VLDL, intermediate density lipoprotein (IDL), and low density lipoprotein (LDL); the IDL and LDL together with chylomicrons remnant recycle back a proportion of the cholesterol to the liver, thus delivering cholesterol from extrahepatic cells to that organ. Therefore, dietary food fortified with vitamin B4 might have benefits to attenuate the atherosclerosis, one of the risk factors of cardiovascular diseases.

Dietary food fortified with vitamin B4 promoted liver phospholipids content. The enhancement of serum lipids content in fasting or starving rats found in the present study indicated that the secretion of those lipids from the liver tissue was stimulated. However, how liver lipid were altered in the liver could not be predicted from serum lipid levels. It was because the lipid level in the liver does not depend only on magnitudes of VLDL secretions from the liver into the bloodstream but also depends on the expression of lipogenic enzymes and genes combined with the level of fatty acids entering into the mitochondrial β-oxidation pathway[15]. As shown in Fig. 2, the liver PL level of Vitamin B4 group increased significantly more than that of the control ($p < 0.05$). The liver TG and cholesterol level, however, were almost similar between the groups.

To clarify which mechanism induces these lipid levels differentiations in liver, the activities of lipogenic and lipolytic enzymes were determined. As shown in Fig. 3, dietary food fortified with vitamin B4 promoted FAS activity by 35%. These data indicated that vitamin B4 stimulated the biosynthesis of fatty acids in liver; however, it ameliorated fatty acid degradation shown by the attenuation of CPT-1 activity (Fig. 3). The increased activities of FAS enzyme indicate that the concentration of fatty acyl-CoA, an activated form of fatty acid, increased.

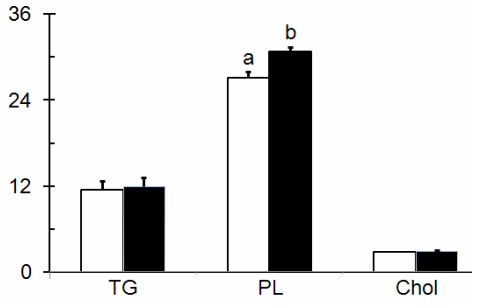

Figure 2. Liver Lipids Content (mg/g liver), for Control Group (□) and Vitamin B4 Group (■)
Rats were paired-fed vitamin B4 supplemented diet or a diet without vitamin B4 (control diet) for 10 days. Rats were killed by decapitation after a 9-h starvation. Values are expressed as mean± SEM of five rats. Clearly define a & b regarding difference of significance at $p < 0.05$.

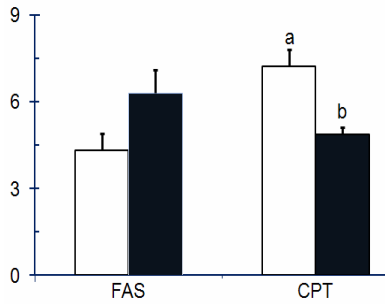

Figure 3. The Activities of Lipogenic and Lipolytic Enzymes (nmol/min/mg Protein), for Control Group (□) and Vitamin B4 Group (■)
Rats were paired-fed vitamin B4 supplemented diet or a diet without vitamin B4 (control diet) for 10 days. Rats were killed by decapitation after a 9-h starvation. Values are expressed as mean± SEM of five rats. Clearly define a & b regarding difference of significance at $p < 0.05$.

The fatty acyl-CoA serves as substrates for several enzymes involved in biosynthesis of several lipid substances including biosynthesis of liver TG, cholesterol, PL, and the degradation of fatty acid following fatty acid β-oxidation. However, the decreases of CPT-1 activity and kept at constant level of liver TG and cholesterol indicated that the promotion of FAS level might be associated with a promotion of liver PL level. The lowered CPT-1 activity, however, indicated that the degradation of fatty acids decreased (Fig. 3). The increased fatty acid biosynthesis might trigger biosynthesis of liver PL and promoted accumulation of PL in liver, which was associated with an increase in its secretion into the bloodstream (Fig. 1).

Conclusion

Dietary food fortified with vitamin B4 attenuates body weight and liver weight but promotes serum lipid and liver PL levels. The induction of hypertriglyceridemia and the promotion of reverse flows of cholesterol from

extrahepatic into liver might indicate a beneficial function of vitamin B4 to regulate the disorders of lipid metabolism in liver and the risk factors of cardiovascular disease. The present study failed to determine the apolipoprotein B100 (ApoB100) secretion and microsomal triglyceride transfer protein (MTP) activity, a key enzyme in VLDL biosynthesis. The ApoB100 secretion and the MTP activity were necessary to be determined in future study.

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