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The Effect of Vitamin B_6 Deficiency on the Utilization of Fuel and Blood Cholesterol Profile with Regular Exercise-Training in Rats

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ABSTRACT

The purpose of this study was to determine whether vitmain B_6(B_6) deficiency affects fuel utilization and blood cholesterol profile with exercise-training. Twenty-four rats were fed a B_6 deficient($-B_6$) diet or a control($+B_6$) diet for 5 weeks and either exercised(EX) or non-exercised(NE). EX rats were exercised on treadmill(10°, 0.5-0.8 km/h) for 20 minutes everyday. Glucose(GLU), glycogen(GLY), protein(PRO), triglyceride(TG), free fatty acid(FFA), total cholesterol(TC), HDL-cholesterol(HDL-C) and LDL-choleterol(LDL-C) were compared in plasma(P), liver(L) and skeletal muscle(M) of rats.

There was a vitamin effect on the level of P-GLU, P-TG, M-TG, L-GLY, L-PRO and an exercise effect on the level of P-PRO, P-FFA, M-PRO, L-GLY, L-TG, P-TC, P-HDL-C, P-LDL-C. Compared to $+B_6$ rats, in NE group, the level of L-GLY of $-B_6$ rats was higher, M-TG and M-PRO of $-B_6$ rats were lower and there were no differences in P-GLU, P-FFA, P-TG. M-GLY, L-TG, P-TC and P-HDL-C. In EX group, the level of P-TG was higher and M-PRO was lower in $-B_6$ rats. There were no defferences in M-GLY, L-TG, P-TC and P-HDL-C.

These results suggest that a lowered intake of vitamin B_6 may impair the adaptation of animals to fuel metabolism related to a decrease of fatty acid oxidation and attenuates the exercise-traning effect on blood lipid profile. (*Korean J Nutrition* 29(8) : 881~888, 1996)

KEY WORDS : vitamin B_6 deficiency · exercise-training · fuel utilization · blood cholesterol profile.

Introduction

Regular exercise has been associated with variable effects on the blood lipid profile as well as on the fuel utilization. Exercise increases energy expenditure. The contribution of the two major fuels, fat and carbohydrate to energy production during exercise depends on various factors including intensity and duration of exercise, state of physical training and diet in healthy state[1]. Training stimulates the muscle cells to manufacture more and larger mitochondria, the cellular structures that conduct aerobic metabolism[2]. Aerobic exercise increases HDL-cholesterol in moderately exercised(45 min. walking at 60% heart rate reserve, five times/wk) obese women[3] as well as in heavily(3500 kcal expenditure/wk) men[4]. However, aerobic exercise had little or no effect on total cholesterol, triglyceride and LDL-cholesterol unless combined with body weight losses in obese men and women[3)5)] and in a meta-analysis of 95 studies[6].

It has been reported indirectly that vitamin B_6 may

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be involved in this fuel metabolism. Pyridoxal 5'-phosphate(PLP), active form of vitamin B_6 acts as an integral parts of glycogen phosphorylase(EC. 2.4.1.1.)[7] which catalyzes the breakdown of glycogen. PLP is also required in biosynthesis of carnitine[8] which acts as a carrier of fatty acyl group across the mitochondrial membrane. PLP is a cofactor for aminotransferase which catalyze the conversion of certain amino acid to glucose[9]. The relationship between PLP and cholesterol remains controversial. It has been also reported that PLP concentration increase during exercise and decrease after exercise [10-12]. However, the direct evidence which vitamin B_6 deficiency affect the body fuel metabolism during chronic exercise has not been reported.

The goals of this study were as followes : 1) to determine whether vitamin B_6 deficiency affects fuel utilization with exercise-training 2) to determine whether vitamin B_6 deficiency will lead to a less desirable blood lipid profile with exercise-training.

Materials and methods

1. Diet and Exercise

Twenty four weanling male Sprague-Dawley rats of 55-65 g were divided into 4 groups ; pair fed control-non exercise group($+B_6$NE), pair fed control-exercise group($+B_6$EX), vitamin B_6 deficient-non exercise group ($-B_6$NE) and vitamin B_6 deficient-exercise group($-B_6$EX). $+B_6$ diet was the vitamin-free, casein-based semi-synthetic diet which met AIN-76 recommendation[13][14]. The composition of $-B_6$ diet was the same as that of $+B_6$ diet except that vitamin B_6 was not added. These rats were fed for 5 weeks with respective diet. $+B_6$ rats were pair-fed against the intake of $-B_6$ rats to minimize the variation due to the difference of the amount of diet consumption. EX group were exercised on treadmill(10°, 0.5-0.8 km/h) for 20 minutes everyday for 5 weeks.

Animals were sacrificed by decapitation under light ether anesthesia after 16 hours fasting. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to seperate plasma. Liver and skeletal muscle(gastrocnemius) was rapidly removed. Plasma and tissues were stored at -40℃ until analyzed.

2. Biochemical and Statistical analysis

Plasma glucose and total cholesterol(TC) was analyzed with commercial kit based on enzymatic method, respectively(Youngdong Pharmaceutical Co., Korea). Triglyceride(TG) was analyzed with commercial kit based on Trinder method(Youngdong Pharmaceutical Co., Korea). Protein was analyzed with commercial kit based on Biuret method(Youngdong Pharmaceutical Co., Korea). Free fatty acid(FFA) was analyzed with commercial kit utilizing acyl CoA synthetase-Acyl CoA oxidase(NEFAZYME-S, Eiken Chemical Co., Japan). HDL-cholesterol(HDL-C) was analyzed with commercial kit based on same analytical method as total cholesterol, after the precipitation of LDL, VLDL and chylomicron with polyethyleneglycol(International Reagent Co., Japan). Tissue samples were homogenized in cold sodium phosphate buffer(0.02M, pH 7.0). Aliquots of the tissue homogenates were analyzed as the same method as that of plasma. Liver and muscle glycogen was measured by a colorimetric procedure[15]. LDL-cholesterol(LDL-C) was estimated by the method of Friedwald et al[16].

All data were subjected to an analysis of variance and tested for significant differences by Duncan's multiple range test[17]. A p value < 0.05 was considered to be significant.

Results

At week 5, the mean body weights of the $-B_6$ rats (NE, 140±17g ; EX, 140±13g) was significantly lower than those of the $+B_6$ rats(NE, 164±5g ; EX, 162±2g) although they were pair-fed. The feed efficiency ratios of the -B6 rats(NE, 0.25±0.04 ; EX, 0.26±0.04) were also significantly lower than those of +B6 rats(NE, 0.29±0.02 ; EX, 0.30±0.01).

The effect of vitamin B_6 deficiency on plasma glucose, protein, free fatty acid and triglyceride with exercise-training is shown in Fig. 1. There was a vitamin effect on the plasma level of glucose($+B_6$; 107.3±7.8, $-B_6$; 87.7±5.5, mg/dl) and triglyceride($+B_6$; 39.3±6.1, $-B_6$; 48.5±6.4, mg/dl) and was an exercise effect on the plasma level of protein(NE ; 8575±831, EX ; 9120±626, mg/dl) and free fatty acid(NE ; 75.3±17.1, EX ; 67.4±19.8, μEq/dl) with exercise-training. In NE group, there was no difference in plas-

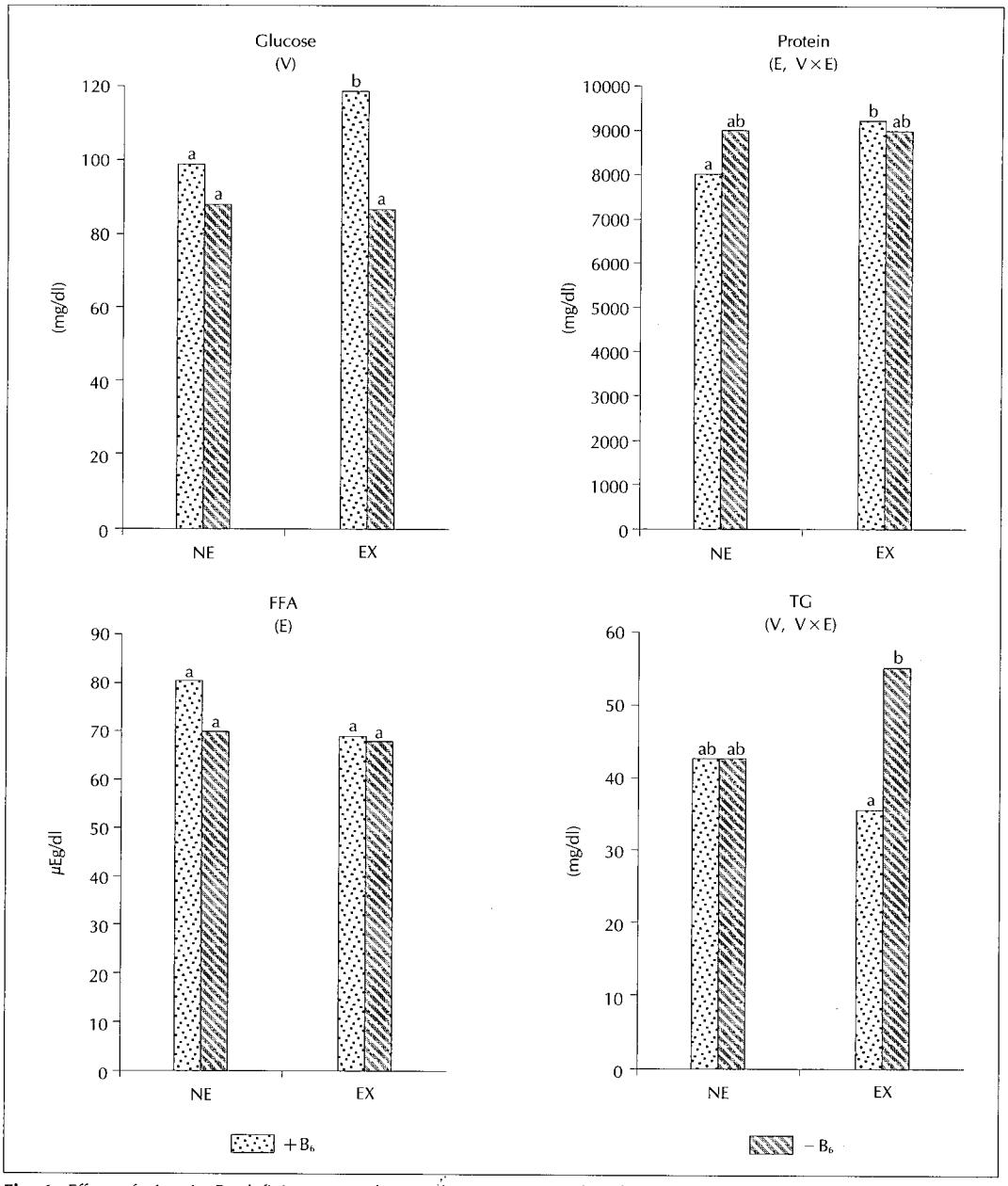

Fig. 1. Effect of vitamin B_6 deficiency on plasma glucose, protein, free fatty acid(FFA), triglyceride(TG) with exercise training. Experimental diets($+B_6$ and $-B_6$) were fed and exercised groups were exercised for 20min/day for 5weeks. Each bar with different letters is significantly different ($p < 0.05$) : $+B_6$=control diet pair fed to $-B_6$ group ; $-B_6$=vitamin B_6 deficient diet ; NE=non-exercise ; EX=exercise ; V=vitamin effect ; E=exercise effect ; V×E=interaction effect between vitamin and exercise.

ma glucose, protein, free fatty acid and triglyceride between $+B_6$ and $-B_6$ rats. In EX group, compared to +B6 rats, the level of plasma glucose was lower and triglyceride was higher in $-B_6$ rats.

Fig. 2 shows the effect of vitamin B_6 deficiency on glycogen, triglyceride, and protein in muscle with exercise-training. There was a vitamin effect on triglyceride level($+B_6$: 2.96±0.57, $-B_6$: 2.15±0.55, mg/g) and an exercise effect on protein level(NE : 225.3±3.6, EX : 208.9±26.8, mg/g) of muscle. In NE group, there was no differences in glycogen and protein between $+B_6$ rats and $-B_6$ rats while triglyceride

level of $-B_6$ rats was significantly lower than that of $+B_6$ rats. In EX group, there was no significant difference in the level of glycogen, triglyceride while protein level of -B6 rats was significantly lower than that of $+B_6$ rats.

Fig. 3 showes the effect of vitamin B_6 deficiency on glycogen, triglyceride and protein in liver with exercise-training. There was a vitamin effect on the level of glycogen($+B_6$; 275.5±59.9, $-B_6$; 469.8±99.7, μg/g) and protein($+B_6$; 310.4±38.4, $-B_6$; 361.3±33.3, mg/g). There was also an exercise effect on glycogen(NE ; 470.5±188.9, EX ; 236.5±93.1, μg/g) and triglyceride(NE ; 21.5±5.6, EX ; 19.2±5.5, mg/g). In NE group, compared to $+B_6$ rats, the level of glycogen was significantly higher and protein tended to be higher in $-B_6$ rats while there was no difference in triglyceride level between $+B_6$ rats and $-B_6$ rats. In EX group, the level of glycogen and pro-

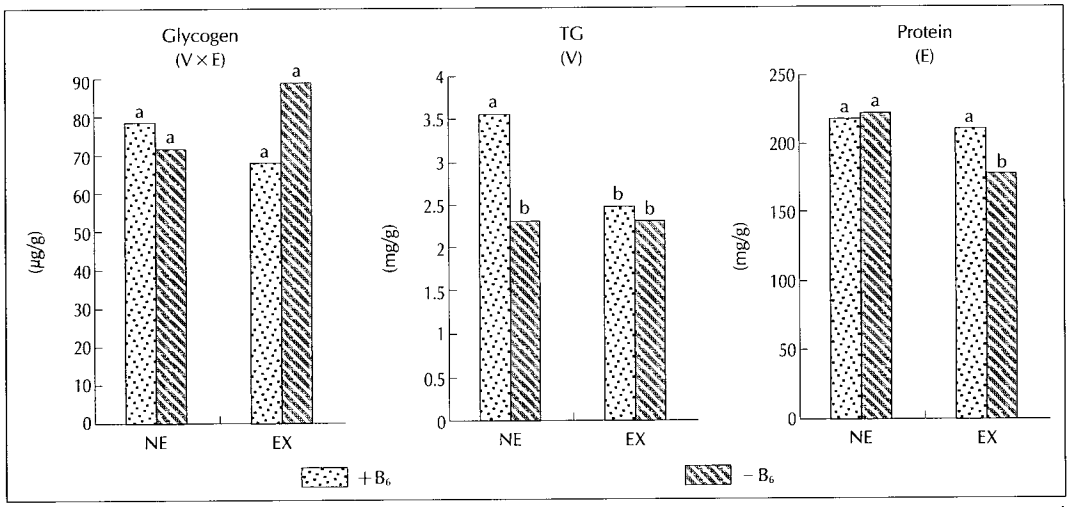

Fig. 2. Effect of vitamin B_6 deficiency on muscle glycogen, triglyceride(TG), protein with exercise training. Experimental diets($+B_6$ and $-B_6$) were fed and exercised groups were exercised for 20min/day for 5weeks. Each bar with different letters is significantly different($p < 0.05$) : $+B_6$=control diet pair fed to $-B_6$ group ; $-B_6$=vitamin B_6 deficient diet ; NE=non-exercise ; EX=exercise ; V=vitamin effect ; E=exercise effect ; V×E=interaction effect between vitamin and exercise.

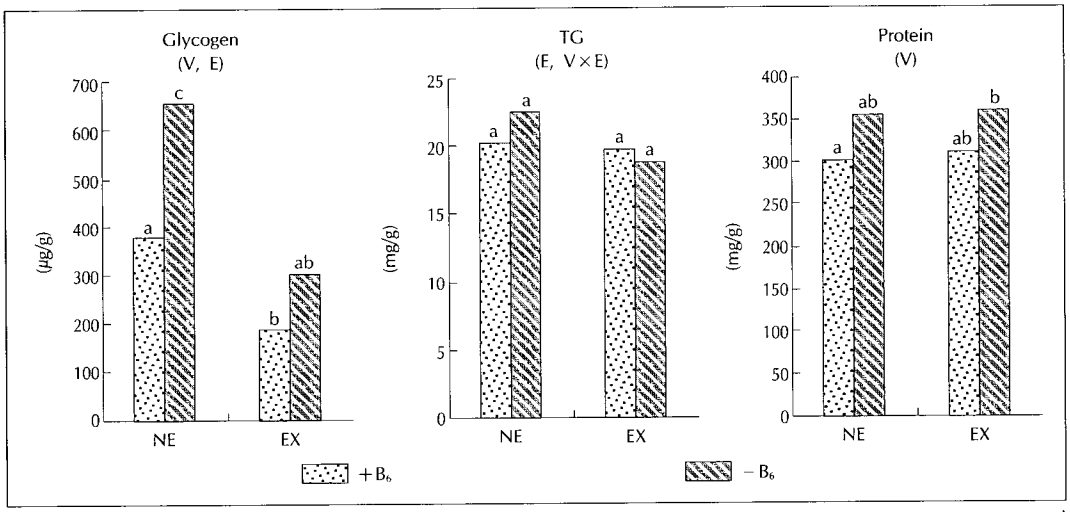

Fig. 3. Effect of vitamin B_6 deficiency on liver glycogen, triglyceride(TG), protein with exercise training. Experimental diets($+B_6$ and $-B_6$) were fed and exercised groups were exercised for 20min/day for 5weeks. Each bar with different letters is significantly different($p < 0.05$) : $+B_6$=control diet pair fed to $-B_6$ group ; $-B_6$=vitamin B_6 deficient diet ; NE=non-exercise ; EX=exercise ; V=vitamin effect ; E=exercise effect ; V×E=interaction effect between vitamin and exercise.

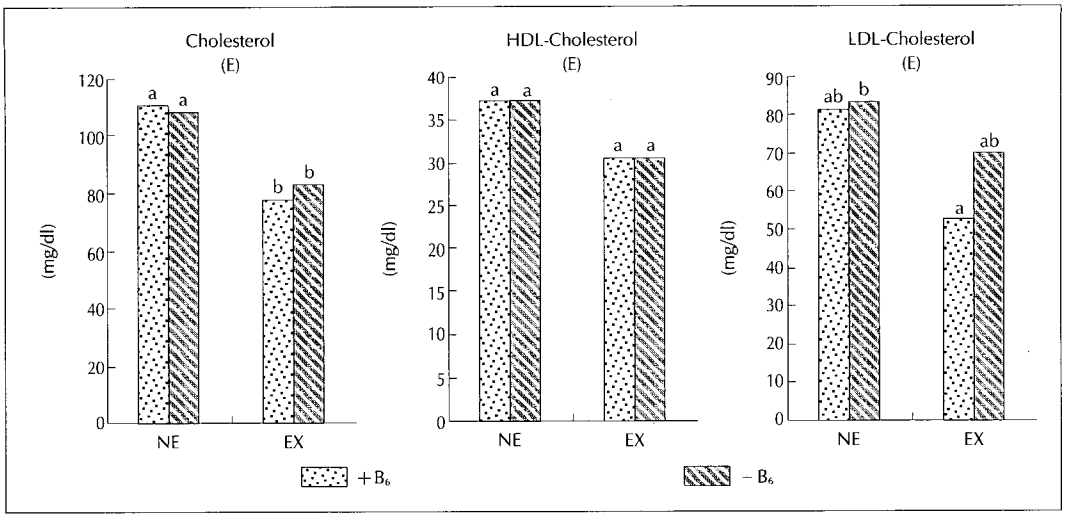

Fig. 4. Effect of vitamin B_6 deficiency on plasma cholesterol, HDL-cholesterol, LDL-cholesterol with exercise training. Experimental diets($+B_6$ and $-B_6$) were fed and exercised groups were exercised for 20min/day for 5weeks. Each bar with different letters is significantly different($p < 0.05$) : $+B_6$=control diet pair fed to $-B_6$ group ; $-B_6$=vitamin B_6 deficient diet ; NE=non-exercise ; EX=exercise ; V=vitamin effect ; E=exercise effect ; V×E =interaction effect between vitamin and exercise.

tein tended to be higher in $-B_6$ rats while there was no difference in triglyceride level between $+B_6$ rats and $-B_6$ rats.

The effect of vitamin B_6 deficiency on plasma total cholesterol, HDL-cholesterol and LDL-cholesterol with exercise-training is shown in Fig. 4. There was only an exercise effect on plasma cholesterol profile. With exercise, the level of total cholesterol was significantly decreased(NE : 110.3±21.4, EX : 80.2±12.3, mg/dl) and LDL-cholesterol tended to be decreased(NE : 84.4±24.5, EX : 80.2±12.3, mg/dl) regardless of vitamin B_6 deficiency.

Discussion

Compared to pair-fed control rats, the lower growth rates and feed efficiency ratios accompanied with clinical deficiency symptoms were shown in $-B_6$ rats. Thus, it was considered that $-B_6$ rats were to be deficient in vitamin B_6 by the 5th week. Since during exercise, glucose output from the liver increases and glucose production through gluconeogenesis normally is not increased[18], the higher level of plasma glucose is thought to be derived from liver glycogen in EX group of $+B_6$ rats. The reason for not having higher plasma glucose level in Ex group of $-B_6$ rats might be either the increase of glucose release from the plasma to the other tissue or the decrease of glucose uptake from the liver. Since the rates of liver glycogen decrease due to exercise were similar between $+B_6$ rats and $-B_6$ rats, it can be assumed that although glucose uptake from the liver increases, the epididymal fat pads from vitamin B_6 deficient rats are more permeable to glucose[19] and the uptake of glucose into fats cell would be increased, resulting in no change in plasma glucose level of $-B_6$ rats. It is reported that, trained muscles store more glycogen within each cell than untrained muscles[20][21] and the changes in body composition depend on more on total energy expenditure than on the type of exercise[22]. Thus, the reason for not having higher muscle glycogen in EX group is assumed that either lower utilization of blood glucose was compensated by the use of muscle glycogen or 20 minutes training in this study might not be enough to adapt to store greater amount of glycogen.

It is reported that muscle's ability to oxidize fat has been thought to be limited by carnitine palmitoyltransferase activity for transport of fatty acid across the mitochondrial membrane[23] and vitamin B_6 is required in carnitine biosynthesis[8]. Thus, the reason for not having different levels of free fatty acid between $+B_6$ and $-B_6$ rats is assumed that the daily 20 minutes exercise, the state of relatively less energy need, was not

long to need fuel from fat oxidation since at low intensities, the fat oxidation increased as exercise time is increased[24]. Another important form of fat for oxidation by muscle during exercise is intramuscular triglyceride[25]. Plasma triglyceride is a potential source of energy for muscle and is important for recovering intramuscular triglyceride during long periods between exercise bouts[26]. Thus, the muscle and plasma triglyceride of exercising rats showed lower level in $+B_6$ rats. However, in $-B_6$ rats, the muscle triglyceride level was not changed and plasma triglyceride was even elevated with exercise training . The reason of this discrepancy might be the result from the impaired fat oxidation due to the vitamin B_6 deficiency. Because exercise leads net loss of vitamin B_6 result from the urinary increase of 4 pyridoxic acid[11], exercise will aggravate vitamin B_6 deficiency in $-B_6$ rats. Thus, the deteriorative effect on plasma triglyceride level showed in $-B_6$ rats with exercise is important since exercise as well as caloric restriction is helpful for the hypertriglyceridemic patient including coronary heart disease[27].

Although exercise training increase the muscle protein in general, muscle protein should be utilized for energy if fat utilization was impaired in $-B_6$ rats. Thus, compared to $+B_6$ rats, with exercise-training, the protein level of $-B_6$ rats was lower in muscle and tended to be lower in plasma. The implication of higher liver protein level of $-B_6$ rats found in this study is unclear. It is possible that amino acid released from muscle protein was utilized for protein synthesis in liver. Moderate exercise-training has been reported to increase HDL-cholesterol[4][5], however, the results of the present study did not support that of others that reported the predomenant effect of exercise-training. Because it is reported that weight loss per se increase HDL-C[4], this difference might be from the differences in initial body weight of the animals or subjects. The body weights of aminals in the present study were in normal range and subject in the above reports [4][5] were obese.

Since total cholesterol level significantly decreased and LDL-cholesterol level tended to be decreased with exercise-training in both $+B_6$ rats and $-B_6$ rats, it can be concluded that exercise-training improves the blood cholesterol profile but vitamin B_6 deficiency have little effect on blood cholesterol profile generally. However, LDL-cholesterol level tended to be higher in both EX group and NE group of $-B_6$ rats compared to that of $+B_6$ rats and the reason of this higher LDL-cholesterol level mihgt be the result of vitamin B_6 deficiency because plasma PLP level were negatively corelated with LDL-cholesterol and total cholesterol in monkeys[28]. In addition, plasma triglyceride level of $-B_6$ rats was significantly higher with exercise-training. Thus, it is suggested that vitamin B_6 deficiency may attenuate the desirable effect of exercise-training on blood lipid profile.

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=국 문 초 록=

비타민 B_6 부족이 정기적인 운동 훈련시 연료의 이용과 혈액 콜레스테롤 성상에 미치는 영향

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본 연구는 비타민 B_6 부족이 정기적인 운동 훈련시 연료이용과 혈액콜레스테롤 성상에 미치는 영향을 연구하고자 하였다.

흰쥐 24마리를 비타민 B_6가 결핍된 식이($-B_6$) 또는 정상식이($+B_6$)를 5주간 먹이면서 반은 운동을 시키지 않았고 반은 treadmill(10°, 0.5~0.8 km/h)에서 매일 20분간 5주간 운동을 시킨후, 포도당(GLU), 글리코겐(GLY), 단백질(PRO), 중성지방(TG), 유리지방산(FFA), 총콜레스테롤(TC), HDL-콜레스테롤(HDL-C), LDL-콜레스테롤(LDL-C)을 혈장(P), 간장(L), 근육(M)에서 비교하였다.

P-GLU, P-TG, M-TG, L-GLY 및 L-PRO 수준에는 비타민 결핍이 영향을 미쳤으며 P-PRO, P-FFA, M-PRO, L-GLY, L-TG, P-TC, P-HDL-C 및 P-LDL-C수준에는 운동여부가 영향을 미쳤다. $+B_6$ 동물에 비하여 비운동군에서 $-B_6$동물의 L-GLY의 수준이 높았고, M-TG, M-PRO수준은 낮았고, P-GLU, P-FFA, P-TG, M-GLY, L-TG, P-TC와 P-HDL-C의 수준은 유의적인 차이를 보이지 않았다. 운동군에서는 $-B_6$ 동물의 P-TG의 수준은 높았고 M-PRO수준은 낮았으며 M-GLY, L-TG, P-TC와 P-HDL-C 수준은 유의적인 차이를 보이지 않았다.

이러한 결과로 볼때 비타민 B_6의 섭취부족은 지방산 산화와 관련된 연료이용의 적응을 손상시킬 수 있으며 정기적인 운동으로 인한 혈액 지질성상 개선 효과를 감소시킬 수 있을 것으로 추정된다.