The Effect of Vitamin B₆ Deficiency on the Utilization and Recuperation of Stored Fuel in Highly Intense Exercised Rats

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Abstract

The purpose of this study was to investigate the effect of vitamin B_6 deficiency on the utilization and recuperation of stored fuel in exercising rats. Thirty six rats were fed either a vitamin B_6 deficient diet($-B_6$) or a control diet($+B_6$) for 5 weeks, then subdivided into 3 groups : non-exercise group(NE), exercise group (EX), exercise and recuperation group(ER). EX group were exercised on treadmill($10°$, $0.5~0.8$km/h) for 2 hours and ER group were recuperated three days with the respective diet after exercise. Glucose(GLU), glycogen(GLY), protein(PRO), triglyceride(TG) and free fatty acid(FFA) were compared in plasma(P), liver (L) and skeletal muscle(M) of rats. Compared to $+B_6$ rats, in NE group, the level of L-GLY of $-B_6$ rats was higher, M-TG, L-PRO of $-B_6$ rats were lower and there were no differences in P-PRO, P-FFA, P-TG, M-GLY, M-PRO and L-TG. In EX group, the levels of P-FFA, L-PRO of $-B_6$ rats were higher. P-TG, L-TG of $-B_6$ rats were lower and there was no difference in L-GLY. In ER group, the levels of P-GLU, P-PRO, P-TG, L-PRO of $-B_6$ rats were lower and there were no differences in L-GLY, L-TG, M-TG and M-GLY. These results suggest that a lowered intake of vitamin B_6 may impair the recuperation of aminals after exercise related to exercise fuel stores although there is a compensation among stored fuel utilization during exercise.

Key words: vitamin B_6 deficiency, exercise, recuperation, fuel utilization

INTRODUCTION

The metabolic events that occur during exercise provide the energy to the exercising muscle of the body and the energy used for exercise in an animal is derived predominantly from carbohydrate and fat. It has been reported indirectly that vitamin B_6 may be involved in this fuel metabolism. Pyridoxal 5'-phosphate (PLP), active form of viatmin B_6 acts as an integral part of glycogen phosphorylase(EC 2.4.1.1.) which catalyzes the breakdown of glycogen(1). PLP is a cofactor for aminotransferase which catalyze the conversion of certain amino acid to glucose(2). PLP is also required in biosynthesis of carnitine which acts as a carrier of fatty acyl group across the mitochondrial membrane(3).

It has been also reported that PLP concentration increase during exercise(4-6). The mechanism of this increase was hypothesized in several ways. Leklem and coworkers(4-6) hypothesized that PLP as a cofactor would be needed in the liver for gluconeogenesis and glycogenolysis. Hofmann et al.(7) proposed that exercising needs more PLP either to facilitate an accelerate rats of aminotransferase, or to saturate the glycogen phosphorylase apoenzyme with PLP. Crozier et al.(8)

postulated that the size in plasma PLP during exercise are more likely a concomitant event accompanying temporary protein shift into blood than an adaptive regulatory event that facilitate fuel provision during exercise. However, the direct evidence that vitamin B_6 deficiency affect the body fuel metabolism during exercise has not been reported.

Thus, the aim of this study was to determine whether fuel metabolism associated with PLP concentration was influenced by vitamin B_6 deficiency during exercise in rats.

MATERIALS AND METHODS

Experimental animals and diets

Thirty six weanling male Sprague-Dawley rats of $40~60$g were fed either vitamin B_6 deficient($-B_6$) diet or control($+B_6$) diet. The control diet was the vitamin-free, casein-based semisynthetic diet which met AIN-76 recommendation(9,10). The composition of $-B_6$ diet was the same as that of control diet except that vitamin B_6 was not added. These rats were fed for 5 weeks with respective diet and then subdivided into 3 groups : non-exercise group(NE), exercise group(EX), exercise

and recuperation group(ER). The +B6 rats were pair-fed against the intake of the -B6 rats to minimize the variation due to the difference of the amount of diet consumption.

Exercise and sample collection

EX group were exercised on treadmill(10°, 0.5~0.8 km/h) for 2 hours and ER group were recuperated three days with the respective diet after exercise. At the respective time points(non-exercise, right after two hours exercise, 3 days recuperation after exercise), animals were sacrificed by decapitation under light ether anesthesia after 16 hours fasting. Immediately following decapitation, blood was collected in heparin-ized tubes and centrifuged to seperate the plasma. The liver and skeletal muscle(gastroenemius) were rapidly removed. The plasma and tissues were stored at -40°C until analyzed.

Biochemical analysis

Plasma glucose was analyzed with a commercial kit based on enzymatic method(Youngdong Pharmaceutical Co., Korea) and triglyceride(TG) was analyzed with a commercial kit utilizing glycerol phosphate oxidase-Quinoneimine coloring method(Youngdong Pharma-ceutical Co., Korea). Protein was measured by Biuret method(11). Free fatty acid(FFA) was analyzed with a commercial kit utilizing acyl CoA synthetase-Acyl CoA oxidase(NEFAZYME-S, Eiken Chemical 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 were mea-sured by a colorimetric procedure(12).

Statistical analysis

All data were subjected to an analysis of variance and tested for significant differences by Duncan's mul-tiple range test(SAS Institute, Cary, NC). A p value< 0.05 was considered to be significant.

RESULTS

Plasma glucose, protein, free fatty acid and tri-glyceride

At week 5, the mean body weight of the -B6 rats(139 ±17g) was significantly lower than that of the +B6 rats(164±5g) and the feed efficiency ratio(FER) of the -B6 rats(0.25±0.04g) was significantly lower than that of +B6 rats(0.29±0.02g). The effect of vitamin B6 de-ficiency on plasma glucose, protein, free fatty acid and triglyceride in exercising rats is shown in Table 1. In the NE group, there was no difference in plasma protein, FFA, TG between +B6 and -B6 rats while the mean concentration of plasma glucose of -B6 rats tended to be lower than that of +B6 rats although this difference was not statistically significant because of the large standard deviation. In the EX group, the mean concen-tration of plasma glucose and FFA of -B6 rats tended to be higher than those of +B6 rats while the mean concentration of plasma TG of -B6 rats tended to be lower than that of +B6 rats. the In the ER group, plasma glucose, protein and TG of -B6 rats were significantly lower than those of +B6 rats.

Table 1. The effect vitamin B6 deficiency on plasma glucose, protein, free fatty acid(FFA), triglyceride(TG)

	+B6			-B6		
	NE	EX	ER	NE	EX	ER
Glucose	97.34[ab1,2]	99.60[ab]	118.62[c]	88.97[a]	106.93[bc]	93.42[ab]
(mg/dl)	±10.12	±10.30	±9.44	±5.45	±12.04	±19.91
Protein	8100.00[a]	8650.00[a]	11080.00[b]	9050.00[a]	8550.00[a]	8933.33[a]
(mg/dl)	±808.29	±597.21	±715.54	±597.21	±854.40	±1604.16
FFA	80.86[a]	88.58[ab]	91.84[ab]	69.75[a]	112.34[b]	67.40[a]
(μEq/dl)	±20.87	±24.97	±24.62	±11.81	±31.49	±15.58
TG	43.10[a]	39.55[ab]	58.10[c]	43.12[a]	30.17[b]	30.58[b]
(mg/dl)	±7.80	±8.31	±4.21	±6.41	±11.31	±7.13

+B6=control diet, -B6=vitamin B6 deficient diet
NE=non-exercise, EX=two hours exercise before sacrifice. ER=two hours exercise and recuperation for three days
[1]Values are mean±S.E., n=5
[2]Within a given row these values with different superscripts are significantly different(p<0.05)

Table 2. The effect of vitamin B-6 deficiency on muscle glycogen, triglyceride(TG), and protein during exercise

	+B6			-B6		
	NE	EX	ER	NE	EX	ER
Glycogen	$77.35^{ab1,2)}$	80.65^a	52.12^c	72.47^{abc}	56.40^{bc}	60.36^{abc}
(μg/g)	±17.32	±25.51	±3.17	±20.73	±6.12	±12.19
TG	3.57^a	3.05^{ab}	3.00^{ab}	2.20^b	2.22^b	2.26^b
(mg/g)	±0.61	±0.85	±0.55	±0.55	±0.72	±1.21
Protein	223.23^{ab}	255.25^a	232.67^{ab}	227.33^{ab}	210.33^{ab}	201.80^b
(mg/g)	±29.39	±42.83	±31.49	±34.67	±21.58	±48.47

+B6=control diet, -B6=vitamin B6 deficient diet
NE=non-exercise, EX=two hours exercise before sacrifice, ER=two hours exercise and recuperation for three days
[1]Values are mean±S.E., n=5
[2]Within a given row these values with different superscripts are significantly different($p<0.05$)

Table 3. The effect vitamin B-6 deficiency on liver glycogen, triglyceride(TG), protein during exercise

	+B6			-B6		
	NE	EX	ER	NE	EX	ER
Glycogen	$360.25^{a1,2)}$	336.32^a	269.73^a	635.75^b	305.93^a	309.38^a
(μg/g)	±61.56	±55.27	±79.99	±200.01	±83.78	±61.92
TG	20.33^{ab}	24.02^a	18.75^{ab}	22.62^{ab}	15.94^b	17.38^{ab}
(mg/g)	±7.04	±5.46	±4.50	±4.19	±5.82	±4.52
Protein	303.83^a	327.33^{ab}	303.67^a	356.67^b	371.00^b	364.00^b
(mg/g)	±41.79	±40.58	±13.72	±33.27	±51.65	±48.27

+B6=control diet, -B6=vitamin B6 deficient diet
NE=non-exercise, EX=two hours exercise before sacrifice, ER=two hours exercise and recuperation for three days
[1]Values are mean±S.E., n=5
[2]Within a given row these values with different superscripts are significantly different($p<0.05$)

Muscle glycogen, triglyceride and protein

Table 2 shows the effect of vitamin B6 deficiency on glycogen, triglyceride and protein in muscle of exercising rats. In the NE group, there was no significant difference in glycogen and protein between +B6 rats and -B6 rats while the level of muscle TG of -B6 rats were significantly lower than that of +B6 rats. In the EX group, the muscle glycogen and protein of +B6 rats were not changed but the muscle glycogen and protein of -B6 rats tended to be decreased. In the ER group, the level of muscle protein was recovered to the level of the NE group in +B6 rats but was not recovered in -B6 rats with 3 days recuperation.

Liver glycogen, triglyceride and protein

The effect of vitamin B6 deficiency on liver glycogen, triglyceride and protein in exercising rats is shown in Table 3. The concentration of liver glycogen and protein was significantly higher than that of +B6 rats in the NE group. This large difference between +B6 and

-B6 rats was lessened during exercise and the difference became insignificant in the EX group or the ER group. In the NE group, there was no significant difference in liver TG between +B6 rats and -B6 rats. However, liver TG level of -B6 rats decreased and showed lower level than that of +B6 rats with exercise. The liver protein level of -B6 rats was higher than that of +B6 rats regardless of exercise.

DISCUSSION

From the lower growth rate and the FER of -B6 rats in spite of pair-feeding with the typical clinical deficiency symptoms of vitamin B6 i.e., characteristic skin lesions and enlarged liver, it was considered that -B6 rats were to be deficient in vitamin B6 by the 5th week.

The major sources of energy for exercise are muscle glycogen, blood glucose, plasma fatty acids and intra-muscular triglyceride(13). Since Ribaya and Gershoff(14) reported that the epididymal fat pads from vitamin B6 deficient rats are more permeable to glucose in the

presence of insulin, the uptake of glucose into fat cells would be increased, resulting in a lower plasma glucose concentration in -B₆ rats, compared to +B₆ rats before exercise. During exercise, blood glucose level of -B₆ rats tended to be higher than that of +B₆ rats and muscle glycogen level of -B₆ rat was lower than that of +B₆ rats. It is reported that muscle glycogen and blood glucose contribute equally to carbohydrate energy production over 2~3 hour of moderate- intensity exercise(15). Therefore, it is assumed that the lower utilization of blood glucose was compensated by the use of muscle glycogen for energy in -B₆ rats.

It is reported that the muscle's ability to oxidize fat has been thought to be limited by carnitine palmitoyl-transferase activity for transport of fatty acids across the mitochondrial membrane(16). Also, FFA binding proteins control the transport of fatty acids through the interstitium and cell membrane as well as cytoplasm(17) and vitamin B₆ is required in carnitine biosynthesis(3). Thus, before exercise, the state of relatively less energy need, plasma FFA concentration between +B₆ rats and -B₆ rats was not significantly different. However, with exercise, the state of expanded energy need and fatty acid oxidation, the impairment of fatty acid oxidation due to vitamin B₆ deficiency might result in a higher plasma FFA level of -B₆ rats than that of +B₆ rats. Another important form of fat for oxidation by muscle during exercise is intramuscular TG (15,18). Plasma TG is a potential source of energy for muscle and is important for recovering intramuscular TG during the long periods between exercise bouts (19). Thus, the muscle TG of -B₆ rats was used for energy as a result of the impaired use of FFA in -B₆ rats and showed lower level of muscle TG as well as plasma TG in -B₆ rats compared to those of +B₆ rats. For the same reason, the liver TG of -B₆ rats might be lower especially during exercise, the state that plasma FFA utilization was severely impaired.

It has been reported that plasma PLP was elevated during endurance exercise(4,6,20). Since PLP is associated with glycogen phosphorylase(1), lowered PLP concentrations could result in impaired glycogenolysis, which resulted in an accumulation of liver glycogen in -B₆ rats before exercise. However, with the elevated plasma PLP and energy needs due to exercise, liver glycogen of -B₆ rats was utilized efficiently and showed no difference in liver glycogen between +B₆ rats and -B₆ rats. Although amino acid metabolism provide 5~10% of energy for sustained exercise(21), muscle protein level was decreased during exercise in both +B₆ rats and -B₆ rats. Also, in -B₆ rats, the level of muscle protein was not recovered after exercise. Thus, it is assumed that muscle protein should be utilized for energy due to the impaired fat utilization in -B₆ rats although severe vitamin B₆ dificiency impaired gluconeogenesis(20). The implication of higher liver protein level of -B₆ rats found in this study is unclear. It is possible that amino acid released from muscle protein was utilized for protein systhesis in liver.

In summary, during exercise, it is assumed that the lower utilization of blood glucose due to vitamin B₆ deficiency was compensated by the use of muscle glycogen and liver glycogen for energy. Also, the impairment of plasma fatty acid oxidation due to vitamin B₆ deficiency was compensated by the use of intramuscular TG for energy and resulted in a higher level of plasma FFA. However, the decreased muscle protein and TG level during exercise was not recovered after exercise. Thus, a lowered intake of vitamin B₆ may impair the recuperation for animal after exercise related to exercise fuel stores and thereby aggravate the health condition although there is a compensation among fuel utilization during exercise.

ACKNOWLEDGEMENTS

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