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The Effects of Vitamin B_6 Deficiency on Stored Fuel Utilization During 3 days Fasting or 6 days Underfeeding in Rats

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ABSTRACT

The effects of vitamin B_6 deficiency on energy utilization during fasting or underfeeding were studied in rats. Fifteen rats were fed a vitamin B_6 deficient(−B6) diet and another 15 rats were fed a control (+B6) diet. These rats were fed for 5 weeks with respective diet, and then subdivided into 3 groups : non-fasted group, fasted group, underfed group. Rats of the fasted group were fasted for 3 days and those of underfed group were fed a half amount of the average consumption of non-fasted group for 6 days. At the respective time (non-fast, 3 day-fast, 6 day-underfeed at 5 weeks), animals were sacrificed.

Feed efficiency ratio of −B6 rats was significantly lower than that of +B6 rats. In −B6 rats, the liver and kidney weights were significantly heavier than those of +B6 rats but spleen and heart weights were not. In non-fasted group, liver protein and triglyceride level of −B6 rats were significantly higher than that of +B6 rats, and muscle protein level of −B6 rats was significantly lower than that of +B6 rats. After −B6 rats were fasted for 3 days, plasma free fatty acid level was significantly lower but liver glycogen level was higher than that of +B6 rats and muscle protein level of +B6 was decreased while that of −B6 was not changed. Vitamin B_6 deficiency had little effect on the energy utilization with 6 days underfeeding.

These results suggest that vitamin B_6 deficiency may impair the utilization of stored fuel during fasting.

KEY WORDS : vitamin B_6 deficiency · fasting · underfeeding · fuel metabolism.

Introduction

The metabolic events that occur during fasting allow animals to live for a period of time in spite of the lack of caloric intake. It has been reported indirectly that vitamin B_6 may be involved in fuel metabolism during fasting. First, vitamin B_6 is required in carnitine biosynthesis in vivo[1]. Carnitine acts as a carrier of fatty acyl group across the mitochondrial membrane for subsequent oxidation. Second, pyridoxal 5'-phosphate(PLP), the biologically active form of vitamin B_6, is a cofactor for aminotransferase, which catalyze the conversion of certain amino acids to glucose[2]. Third, PLP acts as an integral part of glycogen phosphorylase(EC 2.4.1.1.) which catalyzes the breakdown

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of glycogen to glucose 1-phosphate[3]. However, the direct evidence which vitamin B_6 deficiency affect the body fuel metabolism has not been reported.

Therefore, the objective of this research was to investigate the direct evidence of vitamin B_6 deficiency on the concentration of body fuel in vivo.

Materials and Methods

1. Animals and diet

Thirty weanling male Sprague-Dawley rats of 40~60g were fed either vitamin B_6 deficient(−B6) diet or control(+B6) diet. Control diet was the vitamin-free, casein-based semisynthetic diet which met AIN-76 recommendation. The composition of −B6 diet was the same as that of control diet except that vitamin B_6 were not added to the vitamin mixture. These rats were fed for 5 weeks with respective diet and then subdivided into 3 groups : non-fasted group, fasted group, underfed group. +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. Fasted group was fasted for 3 days and underfed group was fed a half amount of the average consumption of non-fasted group for 6 days. Thus, the total diet consumption of fasted group and underfed group was same. At the respective time points(non-fast, 3 day fast, 6 day underfeed at 5 weeks), animals were sacrificed by decapitation under light ether anesthesia after 16 hrs fasting. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to seperate plasma. Organs(liver, heart, spleen and kidneys) were blotted dry and weighed. Skeletal muscle(gastrocnemius) was rapidly removed. Plasma and tissues were stored at −20℃ until analyzed.

2. Biochemical analysis

Plasma glucose concentration was measured by an enzymatic method[4]. Plasma protein was measured by Biuret method[5]. Plasma triglyceride was measured by TRI-25 triglyceride method modified by Giegel et. al.[6] Free fatty acid was measured colorimetrically[7]. Liver and muscle glycogen was measured by a colorimetric procedure[8]. Liver and muscle protein after homogenization was measured by Biuret method. After the liver was homogenized, liver triglyceride was measured by the same method as plasma triglyceride. All data were subjected to an analysis of variance and tested for significant differences by the least significant difference(LSD) test[9]. A p value <0.05 was considered to be significant.

Results

The effect of vitamin B_6 deficiency and the various fasting pattern on body weight and feed efficiency ratio(FER) are shown in Table 1 and 2. At week 5, the mean body weight of the −B6 rats was lower than that of +B6 rats and the FER of −B6 rats was significantly lower than that of +B6 rats. In +B6 rats, the body weights of fasted

Table 1. Effect of vitamin B_6 deficiency on body weight(g) and feed efficiency ratio(FER)

	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	FER
+B6	60.9± 5.0	95.5± 3.3	117.3± 6.8	136.5± 7.6	145.8± 8.0	152.9± 3.6[a]	0.33± 0.02[a]
−B6	61.9± 5.3	102.0± 8.1	120.6± 8.5	138.7± 14.6	148.1± 17.3	138.8± 17.4[b]	0.27± 0.03[b]

1) +B6=control diet, −B6=vitamin B_6 deficient diet

2) Values are mean± S.D., n=15.

3) Within a given column, those values with different superscripts are significantly different($P<0.05$).

Table 2. Effect of vitamin B_6 deficiency on body weight(g) during 3 days fasting or 6 days underfeeding in rats

	+B6[1]	−B6
Non-fasted	152.9± 3.6[a]	138.8± 17.4[a]
Fasted	131.9± 12.0[b]	124.0± 11.8[a]
Underfed	133.4± 8.7[b]	123.5± 13.4[a]

1) +B6=control diet, −B6=vitamin B_6 deficient diet
2) Values are mean S.D., n=15 for non−fasted group, n=5 for fasted and underfed group.
3) Within a given column, those values with different superscripts are significantly different ($P<0.05$).

group and underfed group were significantly lower than that of non-fasted group but in −B6 rats, there was no significant difference among 3 groups. Table 3 shows the effect of vitamin B_6 deficiency on organ weights during fasting or underfeeding in rats. The weight of liver and kidney was significantly increased in −B6 rats. There was no difference in heart and spleen between +B6 and −B6 rats.

The effect of vitamin B_6 deficiency on plasma glucose, protein, triglyceride and free fatty acid

Table 3. Effect of vitamin B6 deficiency on organ weights during fasting or underfeeding in rats

	Non-fasted		Fasted		Underfed	
	+B6[1]	−B6	+B6	−B6	+B6	−B6
Liver(g)	4.43± 0.39[b]	5.54± 0.87[a]	3.09± 0.20[cd]	3.95± 0.73[cd]	3.20± 1.78[d]	3.64± 0.68[bc]
(g/100g BW)	2.90± 0.23[bc]	4.01± 0.57[a]	2.32± 0.17[c]	3.23± 0.74[b]	2.44± 2.92[c]	2.92± 0.41[b]
Kidney2(g)	0.67± 0.04[bc]	0.94± 0.07[a]	0.61± 0.04[bc]	0.86± 0.16[a]	0.59± 0.06[c]	0.71± 0.04[b]
(g/100g BW)	0.44± 0.02[c]	0.68± 0.05[a]	0.46± 0.00[c]	0.70± 0.07[a]	0.45± 0.05[c]	0.57± 0.05[b]
Heart(g)	0.50± 0.04[a]	0.49± 0.07[a]	0.42± 0.03[bc]	0.46± 0.06[ab]	0.41± 0.02[bc]	0.37± 0.02[c]
(g/100g BW)	0.33± 0.02[b]	0.35± 0.04[ab]	0.32± 0.04[b]	0.37± 0.03[a]	0.31± 0.03[b]	0.30± 0.02[b]
Spleen(g)	0.42± 0.22[a]	0.34± 0.08[ab]	0.24± 0.02[b]	0.30± 0.08[ab]	0.29± 0.11[ab]	0.38± 0.11[ab]
(g/100g BW)	0.27± 0.11[ab]	0.25± 0.06[ab]	0.18± 0.02[b]	0.24± 0.06[ab]	0.23± 0.10[ab]	0.31± 0.02[a]

1) +B6=control diet, −B6=vitamin B_6 deficient diet
2) Means of two kidneys
3) Values are mean± S.D., n=5.
4) Within a given row, those values with different superscripts are significantly different($P<0.05$)

Table 4. Effect of vitamin B_6 deficiency on plasma glucose, protein, triglyceride(TG) and free fatty acid(FFA) during fasting or underfeeding in rats

	Non-fasted		Fasted		Underfed	
	+B6[1]	−B6	+B6	−B6	+B6	−B6
Glucose (mmol/L)	6.55± 1.39[a]	6.66± 0.17[a]	5.18± 0.28[b]	5.15± 0.85[b]	5.06± 0.24[b]	5.25± 0.35[b]
Protein (g/L)	145.3 ± 29.4[a]	151.7 ± 12.5 [a]	119.0 ± 18.1[b]	122.5 ± 10.4[b]	122.1± 3.2[b]	116.7 ± 6.9[b]
TG (mmol/L)	0.34± 0.16[a]	0.30± 0.13[a]	0.36± 0.13[a]	0.35± 0.18[a]	0.22± 0.06[a]	0.24± 0.11[a]
FFA (mg/L)	161± 8[b]	165± 4[b]	226± 26[a]	117± 36[c]	142± 28[bc]	167± 6[b]

1) +B6=control diet, −B6=vitamin B_6 deficient diet
2) Values are mean S.D., n=5
3) Within a given row, those values with different superscripts are significantly different ($P<0.05$).

during fasting and underfeeding is shown in Table 4. In non-fasted group and underfed group, there was no significant difference in plasma glucose, protein, triglyceride between −B6 and +B6 rats. In fasted group, the mean concentration of plasma free fatty acid of −B6 rats was significantly($p<0.05$) lower than that of +B6 rats.

Table 5 shows the effect of vitamin B_6 deficiency on liver glycogen, protein and triglyceride during fasting or underfeeding. Liver triglyceride concentration of −B6 rats was significantly higher than that of +B6 rats in non-fasted group. This large difference between +B6 and −B6 rats in non-fasted group is lessened on fasting or underfeeding and the difference became insignificant in fasted and underfed group. There was no difference in liver protein between +B6 and −B6 rats of all group. Liver glycogen concentration of −B6 rats is higher than that of +B6 rats in fasted group while there was no difference in liver glycogen between +B6 and −B6 rats in non-fasted group and underfed group.

The effect of vitamin B_6 deficiency on muscle glycogen and protein during fasting or underfeeding is shown in Table 6. Neither vitamin B_6 deficiency nor feeding type affected the muscle glycogen concentration. Muscle protein concentration of −B6 rats was significantly lower than that of +B6 rats in non-fasted group. After 3 days fasting, muscle protein level of +B6 rats was decreased while that of −B6 rats was not changed and thereby this difference became insignificant in fasted group and underfed group.

Table 5. Effect of vitamin B_6 deficiency on liver glycogen, protein and triglyceride(TG) during fasting or underfeeding in rats

	Non-fasted		Fasted		Underfed	
	+B6[1]	−B6	+B6	−B6	+B6	−B6
Glucose (μg/g)	226.7 ± 108.7[b]	305.9± 167.9[ab]	186.5± 65.2[b]	364.0± 135.5[a]	222.0± 23.5[b]	233. 9± 44.6[ab]
Protein (mg/g)	230.8 ± 13.4[b]	290.1± 89.6[a]	222.1± 10.1[b]	273.9± 51.2[ab]	233.2± 13[ab]	247. 3± 25.2[ab]
TG (μmol/g)	3.39± 2.8[b]	123.1± 102.8[a]	2.39± 0.2[b]	3.80± 1.9[b]	1.48± 0.3[b]	2.60± 1.4[b]

1) +B6=control diet, −B6=vitamin B_6 deficient diet
2) Values are mean± S.D., n=5
3) Within a given row, those values with different superscripts are significantly different($P<0.05$)

Table 6. Effect of vitamin B_6 deficiency on muscle glycogen and protein during fasting or underfeeding in rats

	Non-fasted		Fasted		Underfed	
	+B6[1]	−B6	+B6	−B6	+B6	−B6
Glucose (μg/g)	129.4± 60.7[a]	106.0± 35.3[a]	122.1± 29.1[a]	104.4± 29.3[a]	130.8± 48.0[a]	118.7± 36.8[a]
Protein (mg/g)	275.9± 24.8[a]	235.4± 10.7[b]	225.1± 11.7[b]	237.4± 29.7[b]	210.5± 10.0[b]	226.8± 22.5[b]

1) +B6=control diet, −B6=vitamin B_6 deficient diet
2) Values are mean± S.D., n=5
3) Within a given row, those values with different superscripts are significantly different ($P<0.05$)

Discussion

At various points during the study, the vitamin B_6 status of the rats was evaluated using body weight and FER as a long-term measure. From lower growth rate and the FER of −B6 rats inspite of pair feeding to +B6 rats accompanied with the typical clinical deficiency symptoms of vitamin B_6, ie, characteristic skin lesions and enlarged liver, it was considered that −B6 animals were to be deficient in vitamin B_6 by the 5th week.

In relation to body weight, the weight of liver and kidney was significantly increased in −B6 rats of non-fasted group. This enlarged liver is in agreement with that of our previous study[10]. Because the concentration of liver triglyceride was higher in −B6 rats of non-fasted and fasted groups, this enlarged liver is assumed mainly due to triglyceride in the liver. The support for this comes from the underfeeding study[11-15]. After −B6 rats was underfed, the liver triglyceride level is decreased and the weight of liver returned to the level of +B6 rats.

Since fasting state needs more utilization of stored fuel, the release of free fatty acid from adipose tissue is important for living. In fasting state, compared to +B6 rats, −B6 rats had significant lower free fatty acid concentration, which was also observed in other vitamin B_6 deficient study[16]. The low free fatty acid values reflect either lower stores or impaired release from adipose tissue. Since triglyceride of liver and plasma of +B6 rats was higher or was not different from that of +B6 rats, the lowered free fatty acid concentration in plasma suggest that the release of free fatty acid from adipose tissue into the plasma may be impaired as a result of vitamin B_6 deficiency.

Another important source of energy during fasting is glycogen of liver and muscle. The higher glycogen content in liver of −B6 rats may be due to either an impaired breakdown of glycogen or to an increased synthesis of glycogen. Because PLP acts as an integral part of glycogen phosphorylase(EC 2.4.1.1.) which catalyzes the breakdown of glycogen to glucose 1-phosphate, it can be inferred that vitamin B_6 deficiency may depress the breakdown of glycogen, then progressively accumulate glycogen in the liver. Thus, the difference of liver glycogen between +B6 and −B6 rats became more significant with fasting.

Although the utilization of stored energy is needed during fasting, if liver glycogen and fat from adipose tissue can not be available for energy in vitamin B_6 deficient group, muscle glycogen or protein should be used. However, muscle glycogen of −B6 rats was not different from that of +B6 rats. This result is consistent with the finding of Illingworth et. al.[17] which muscle glycogen content of rats maintained on a pyridoxine deficient diet was not different from the control rats although the total phosphorylase activity of the skeletal muscle of rats maintained on a pyridoxine deficient diet has been found to fall to 35% of the normal value. Also, fasting did not affect the level of muscle glycogen in all rats. In contrast to the observation of the present study, Zorzano et. al.[18] reported a decrease in starved skeletal muscle glycogen. A possible reason for this discrepancy may be the feeding pattern before fasting (ad libitum vs pair-feeding). It is assumed that, with feeding, while there was no need to utilize the muscle protein for energy source in +B6 rats, muscle protein might be already used for energy although some difficulty due to the deficiency of PLP was occured because −B6 rats could not utilize glycogen effectively, and muscle protein concentration of −B6 rats was significantly lower than that of +B6 rats. Also, with fasting, while muscle protein of −B6 rats might be difficult to

be used for energy source due to lack of PLP for aminotransferase, muscle protein in +B6 rats can be easily used for glucose source and muscle protein concentration tends to be decreased. Thus, the difference of muscle protein between two groups became to be insignificant in fasted group and underfed group.

In conclusion, with 6 days underfeeding, vitamin B_6 deficiency did not affect the fuel utilization while with 3 days fasting, the effects of vitamin B_6 deficiency on fuel utilization was evident although the total food consumption of fasted group and underfed group was same. Considering the physiological importance of the utilization of stored fuel during fasting, the difficulty with the use of glycogen, free fatty acid, or protein results in a decrease of an available fuel source. Thus, a lowered intake of vitamin B_6 may impair the adaptation of animals during fasting related to fuel utilization and thereby aggravate the health condition.

Literature cited

1) Cho Y and Leklem JE. In vivo evidence for a vitamin B_6 requirement in carnitine synthesis. *J Nutr* 120 : 258-265, 1990
2) Plebani M, Pesarin F, Ceriotti G. Reference values for alanine and aspartate aminotransferase(ALT and AST) optimized by addition of pyridoxal phosphate. *Enzymes* 25 : 346-352, 1980
3) Krebs EG, Fischer EH. Phosphorylase and related enzymes of glycogen metabolism. *Vitamin Hormone* 22 : 399-410, 1964
4) Pileggi VJ, Szustkrewixz CP. Carbohydrates. In : Henry RJ, ed. Clinical chemistry principles and techniques, pp1265-1295, Haper and Row, Hagerstown MD, 1974
5) Gornall AG, Bardawill CS, David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 177 : 751-766, 1949
6) Folch J, Lees M, Soane-Stanley GH. A simple method for the isolation and purification of total lipid from animal tissues. *J Biol Chem* 226 : 497-509, 1957
7) Falholt K, Lund B, Falholt W. An easy colorimetric micromethod for routine determination of free fatty acids in plasma. *Clin Chim Acta* 466 : 105-111, 1973
8) Hassid WZ, Abraham X. Chemical procedures for analysis of polysaccharides. In : Coluwick SP, ed. Methods in enzymology vol III, pp34-50, Academic press, 1957
9) Heintz JL. Number cruncher's statistical system for IBM-PC users, Kasville, Utah, 1986
10) Yu JY, Cho Y. Tissue iron content in riboflavin and pyridoxine deficient rats. *J Nutr Biochem* 1 : 310-314, 1990
11) Abe M. Kishino Y. Pathogenesis of fatty liver in rats fed a high protein diet without pyridoxine. *J Nutr* 112 : 205-210, 1982
12) Suzuki K, Okada M. Alterations of phospholipid and triglyceride metabolism in fatty liver caused by pyridoxine deficiency in rats. *J Nutr Sci Vitaminol* 28 : 377-390, 1982
13) Kishino Y, Abe M. Lysosomal changes in fatty liver induced in rats by high protein pyridoxine-deficient diet. *J Nutr Sci Vitaminol* 28 : 209-216, 1982
14) Dussault PE, Lepage M. In vitro studies of fatty acid metabolism in vitamin B_6 deficient rats. *J Nutr* 109 : 138-141, 1979
15) Suzuki K, Nakamura T, Fujita M, Iwami T, Abe M, Okada M. Factors affecting liver lipid content in pyridoxine-deficient rats. I. Dietary protein levels. *J Nutr Sci Vitaminol* 22 : 291-298, 1976
16) Audet A, Lupien PJ. Triglyceride metabolism in pyridoxine-deficient rats. *J Nutr* 104 : 91-100, 1974
17) Illingworth B, Kornfeld R, Brown DH. Phosphorylase and uridinediphosphoglucose-glycogen transferase in pyridoxine deficiency. *Biochim Biophys*

Acta 42 : 486-489, 1960

18) Zorzano A, Balon TW, Brady LJ, Rivera P, Geretto LP, Young JC, Goodman MN, Ruderman NB. Effects of starvation and exercise on concentrations of citrate, hexose phosphates, and glycogen in skeletal muscle and heart. *Biochem J* 232 : 585-591, 1985

= 국문초록 =

Vitamin B_6 결핍이 3일간 금식 또는 6일간 감식 흰쥐의 에너지대사물 농도에 미치는 영향

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비타민 B_6의 결핍이 금식 또는 감식하는 동안 흰쥐의 저장에너지 이용에 미치는 영향을 연구하였다. 15마리는 비타민 B_6가 결핍된 식이(−B6)를 먹였고 다른 15마리는 정상식이(+B6)를 먹였다. 이 쥐들을 각각의 식이로 5주간 먹인뒤 3개의 소그룹 : 비금식군, 금식군, 감식군으로 나누었다. 금식군은 3일간 금식시켰으며, 감식군은 비금식군의 평균섭취량의 반을 6일동안 먹였다. 동물들은 각 시점(금식시키기전, 3일간 금식시킨후, 6일간 감식시킨후)에서 희생시켰다.

−B6군의 식이이용효율은 +B6군보다 유의적으로 낮게 나타났다. −B6군의 간과 신장의 무게는 +B6군보다 유의적으로 무거웠으나, 비장과 심장의 무게는 차이가 없었다. 비금식군에서 −B6군의 간장의 단백질 함량과 중성지방 함량은 +B6군보다 유의적으로 높게 나타났으며, −B6군의 근육 단백질 함량도 +B6군보다 유의적으로 높게 나타났다. 3일간 금식시킨후에, −B6군의 현장 유리지방산 함량은 +B6군보다 유의적으로 낮게 나타났으나, 간장 글리코겐 함량은 높게 나타났으며, −B6군의 근육 단백질 함량은 변하지 않은데 반해 +B6군에서는 감소되었다. 비타민 B_6의 결핍은 6일간 감식하는 동안의 저장에너지 이용에는 큰영향을 미치지 않았다.

이러한 결과로 볼때 비타민 B_6의 결핍은 금식하는 동안 저장에너지의 이용에 손상을 줄것으로 보인다.