

# Effect of Thiamine Pyrophosphate (Bicarbossilasi®) Administration on the Exercising Horse Metabolism

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## ABSTRACT

Thiamine pyrophosphate (TPP) is the phosphorylated and active form of thiamine (Vitamin B1). We hypothesized that the administration of thiamine in its immediately available active form could provide the metabolic pathways for a supplement able to promote the metabolism of ketoacids and to reduce lactate accumulation in exercising horses. Ten horses were conditioned for 20 days to daily standardized exercise. All horses underwent a first “stress test” (ST) consisting in 1,200 meters at maximum speed on track, and were checked before and after for clinical and clinical pathology parameters. After the ST, the same animals were administered TPP (Bicarbossilasi®, Teknofarma S.p.A., Torino, Italy), 1 mg/Kg b.w., I.V., twice daily for seven days. At the end of treatment, a second stress test (ST<sub>TPP</sub>) was performed. Post-exercise serum lactate concentration resulted in significantly lower levels ( $p < 0.05$ ) after treatment with TPP (ST vs ST<sub>TPP</sub>). These data suggest that supplementation with thiamine in its active form improves glucose metabolism and prevents lactate accumulation in muscle, enhancing aerobic capacity and metabolic pathways of glucose utilization during exercise.

**Keywords:** Horse; Equine; Exercise; Vitamin B; Thiamine; Glucose Metabolism; Lactate.

## INTRODUCTION

Vitamin B plays an important role in energy metabolism and is considered a key nutrient in exercising horses (1). Thiamine pyrophosphate (TPP) is the phosphorylated and activated form of thiamine (Vitamin B1). The activation of thiamine occurs primarily in the liver with ATP consumption, and once phosphorylated, the molecule acquires the active form as TPP. All living organisms need thiamine, but only bacteria, fungi, and plants are able to synthesize it. Thiamine is an essential nutrient for animals and humans, and must be obtained from the diet or through metabolic production by gut microorganisms (2).

TPP mainly acts as coenzyme for the decarboxylation

of ketoacids and plays an essential role in the oxidative decarboxylation of pyruvate, allowing the formation of acetyl coenzyme-A under aerobic condition. The latter will enter in the Krebs cycle to allow for energy production (1). Since pyruvate is derived from glucose, it should be emphasized that the energy drive from glucose oxidation is highly dependent upon TPP (3).

During short and intense efforts, especially in the most energy demanding tissue such as muscles, an excess of pyruvate is produced as the final product of glycolysis and can be converted to lactic acid (4). The excess of lactic acid is transferred into the blood and reaches the liver where it is converted back to pyruvate and then glucose (Cori cycle) (4).

If the effort is continuous, active muscles may fail to transfer the excessive lactic acid into the blood, which contributes to symptoms of “fatigue” due to the alteration of the physiological pH of the cells.

The term “fatigue” indicates a clinical condition of athletic horses causing the interruption of exercise or a reduction in its intensity (5). In case of high-intensity exercise, fatigue is caused by the accumulation of catabolites in blood and muscle which affects the contractile capacity of myocytes (6). It is evident that as the threshold for “fatigue” increases, the improvement in the performance of the horse will increase as well. Studies in human athletes demonstrated that fatigue can be delayed and performance enhanced if blood glucose levels are maintained by oral or intravenous supplementation of glucose (7). Given the crucial involvement of thiamine in glucose metabolism and the aerobic pathway, particularly in the muscles, its importance is evident during exercise and in the onset of “fatigue” (1).

Thiamine deficiency can cause neurologic disorders and thiamine supplementation can relieve symptoms or resolve several neurologic diseases in cats (8, 9), dogs (10), cows (11, 12) and humans (13, 14). Reduction in serum lactate concentration has also been observed in pigs after TPP administration (15). Cocarboxylase was found to be beneficial in the ischemic canine myocardium due to its favorable systemic hemodynamic effects (16).

The effect of thiamine administration has been tested in human athletes with different results. In some studies thiamine was able to reduce serum lactate concentration and to improve resistance to fatigue (17, 18). On the other hand, other surveys did not obtain similar results (19, 20). A previous study in athletic horses has shown that the oral administration of supplemental thiamine decreased the levels of serum lactate during exercise (21).

Thiamine pyrophosphate is the active ingredient of the veterinary drug Bicarbossilasi® (Teknofarma S.p.A., Torino, Italy). In the present study, the effect of thiamine pyrophosphate administration to athletic horses during intense exercise has been tested in a clinical, cross over trial.

## MATERIALS AND METHODS

### Animals

Ten pleasure horses (6 male and 4 female) normally used for recreational riding (e.g. trail riding) were included in the study.

Breeds of horses included four Sella Italiano, three Anglo-Arabian, two Murgese and one Tolfetano. All horses were active at the time of the study. The age of the horses ranged from 4 to 21 year (mean:  $10.6 \pm 6.0$  years), and their weight ranged from 407 to 600 Kg (mean:  $495.2 \pm 57.3$  Kg). All animals, once included in the study, were stabled in boxes and subjected to a standardized diet: hay and water *ad libitum* and integrated with complementary feed corresponding to about 1% of their body weight divided into two daily rations. Clinical assessment was performed daily to check for any symptoms that could cause withdrawing the animals from the study.

### Study design

Before starting the study, the animals were conditioned for 20 days to daily standardized training sessions lasting 35 minutes and consisting of a warming-up phase (walk for 10 minutes), followed by 5 minutes trot, 5 minutes gallop, 5 minutes trot and 10 minutes walk.

At the end of the 20 days, the horses underwent their first stress test (ST): 400 meters walk followed by 800 meters trot; the horses then performed an exercise at their maximum potential (maximum speed) for 1,200 meters. Clinical and clinical pathology evaluations were performed before (T0) and immediately after (T1) the ST. The post-stress evaluations were made directly on the track immediately after each horse had stopped.

Starting from the first day, the administration of TPP was commenced, at the dose of 1 mg/Kg b.w., I.V., twice a day and continued for seven days. At the end of the administration period, the horses underwent a second stress test (ST<sub>TPP</sub>) with the same protocol previous described and with clinical and clinical pathology evaluation as previously performed (at T0<sub>TPP</sub> and at T1<sub>TPP</sub>).

The horses were ridden by the same rider during both ST and ST<sub>TPP</sub>.

### Sampling and clinical pathology evaluations

Venous blood samples were collected from the jugular vein into sterile tubes with and without ethylene diaminetetraacetic acid (EDTA) at T0, T1, T0<sub>TPP</sub> and T1<sub>TPP</sub> and maintained at 4°C until the delivery to the laboratory within 3 hours from collection. The following hematologic and biochemical parameters were measured within 12 hours from sample collection: erythrocytes (red blood cells, RBC), packed cell volume (PCV), hemoglobin (Hb), red cell distribution width

(RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets along with PDWc (platelet distribution width) and MPV (mean platelet volume) (Cell Dyn 3500, Abbott, Chicago, IL, USA).

The biochemical profile included aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatine phosphokinase (CPK), total protein (TP), albumin (Alb), urea, creatinine (Crea), glucose (Glu), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TB), direct bilirubin (DB), triglycerides (Tryg), cholesterol (Chol), calcium, phosphorus, magnesium, sodium, potassium, and chlorine (Targa 3000 plus, Biotechnica Instruments, Italy, Rome).

Blood lactate was analysed using a portable device (Accutrend® Plus, Roche, Mannheim, Germany) at T0, T1, T1+2h, T1+8h, T1+18h, T0<sub>TPP</sub>, T1<sub>TPP</sub>, T1<sub>TPP</sub>+2h, T1<sub>TPP</sub>+8h, T1<sub>TPP</sub>+18h.

## Statistical analysis

All results are expressed as the mean value and standard deviation (SD) for each stage of the test. Data collected at T0 and T1 were compared with those obtained at T0<sub>TPP</sub> and T1<sub>TPP</sub>, respectively, by the Student's t-test. For serum lactate, the comparison was performed between each sampling stage before (T0, T1, T1+2h, T1+8h, T1+18h) and after (T0<sub>TPP</sub>, T1<sub>TPP</sub>, T1<sub>TPP</sub>+2h, T1<sub>TPP</sub>+8h, T1<sub>TPP</sub>+18h) thiamine administration.

P value for statistical significance was set at <0.05 for all tests. Statistical analyses were performed using the WINPEPI (PEPI-for-Windows) computer programs (22).

## RESULTS

None of the horses showed clinical signs that could result in withdrawal from the study. Values for clinical and laboratory data at different stage of the study and normal ranges (23, 24, 25, 26, 27) are reported in Tables 1, 2, and 3.

Statistically significant differences ( $P < 0.05$ ) between T0

**Table 1.** Mean value for haematological parameters before and after the first (ST) and the second (ST<sub>TPP</sub>) stress test.

Parameter	ST		ST <sub>TPP</sub>		Normal range
	T0 Mean (SD)	T1 Mean (SD)	T0 <sub>TPP</sub> Mean (SD)	T1 <sub>TPP</sub> Mean (SD)	
WBC (x10 <sup>3</sup> /μl)	9.5* (2.1)	9.2 (2.1)	8.7* (1.4)	9.5 (1.8)	5.6-12.1
LYM (x10 <sup>3</sup> /μl)	3.3 (2.2)	3.6 (1.9)	3.2 (1.8)	3.3 (1.8)	1.2-5.1
MON (x10 <sup>3</sup> /μl)	0.5 (0.3)	0.5 (0.4)	0.2 (0.2)	0.4 (0.4)	0-0.7
NEU (x10 <sup>3</sup> /μl)	5.1 (1.0)	4.5 (1.2)	4.7 (1.0)	5.2 (1.5)	2.9-8.5
EOS (x10 <sup>3</sup> /μl)	0.6 (0.2)	0.5 (0.2)	0.5 (0.2)	0.6 (0.4)	0-0.8
BAS (x10 <sup>3</sup> /μl)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0-0.3
LYM%	32.8 (13.0)	37.5 (13.2)	35.1 (13.3)	34.2 (15.0)	17-68
MON%	5.7 (3.9)	5.5 (3.1)	3.0 (3.1)	4.0 (3.4)	0-14
NEU%	54.5 (11.6)	50.4 (11.9)	55.1 (10.9)	54.6 (12.2)	22-72
EOS%	6.3 (2.8)	5.9 (2.6)	5.9 (2.8)	6.5 (3.6)	0-10
BAS%	0.8 (0.3)	0.8 (0.4)	0.8 (0.4)	0.8 (0.4)	0-4
RBC (x10 <sup>6</sup> /μl)	8.9 (0.9)	8.8 (0.7)	8.7 (0.7)	9.3 (2.6)	6-10.4
HGB (g/dl)	13.8 (1.6)	13.6 (0.9)	13.8 (1.1)	14.8 (4.9)	11-19
HCT %	40.1 (4.3)	40.2 (3.5)	39.9 (3.0)	43.0 (13.0)	32-53
MCV (fl)	45.4 (2.4)	45.6 (2.6)	45.8 (2.3)	46.1 (2.1)	37-49
MCH (pg)	15.6 (0.7)	15.4 (0.7)	15.9 (0.8)	15.8 (0.8)	13.7-18.2
MCHC (g/dl)	34.5 (1.0)	33.9 (1.5)	34.7 (1.6)	34.2 (1.1)	31.0-38.6
RDWc %	21.1 (0.5)	21.4 (0.6)	21.2 (0.6)	21.6 (0.8)	18-23
PLT (10 <sup>3</sup> /μl)	178.8 (28.9)	182.9 (32.4)	171.8 (40.6)	192.1 (57.1)	117-256
PCT%	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0-0.1
MPV(fl)	7.3 (0.8)	7.5 (0.6)	7.5 (0.5)	7.2 (0.7)	5.4-9.3
PDWc%	34.1* (1.7)	36.8 (1.8)	36.8* (2.3)	36.0 (1.9)	26-70

\*= $P < 0.05$

**Table 2.** Mean value for biochemical parameters before and after the first (ST) and the second (ST<sub>TPP</sub>) stress test.

Parameter	ST		ST <sub>TPP</sub>		Normal Range
	T0 Mean (SD)	T1 (Mean (SD))	T0 <sub>TPP</sub> Mean (SD)	T1 <sub>TPP</sub> Mean (SD)	
AST (U/l)	315.1 (69.8)*	289.9 (33.0) *	266.9 (89.4)*	282.4 (60.2) *	160-412
LDH (U/l)	716.5 (163.0)	631.1 (142.1)	756.7 (153.9)	719.8 (180.7)	162-412
ALP (U/l)	412.8 (176.1)	375.9 (102.9)	362.0 (87.9)	370.3 (83.6)	143-395
GGT (U/l)	23.9 (15.2)	20.9 (10.3)	19.7 (8.0)	20.1 (6.5)	6-32
CK (U/l)	286.6 (111.4)	292.1 (76.4)	521.1 (53.1)	434.3 (39.5)	60-330
BUN (mg/dl)	30.7 (7.8) *	28.4 (7.1) *	21.6 (5.1) *	21.7 (5.3)*	11-27
CREA (mg/dl)	1.1 (0.2)	1.2 (0.2)	1.2 (0.1)	1.3 (0.1)	0.4-2.2
PROT (g/dl)	7.4 (0.3)	7.4 (0.3)	7.4 (0.6)	7.2 (0.5)	5.6-7.6
ALB (g/dl)	3.3 (0.2)	3.4 (0.2)	3.3 (0.2)	3.2 (0.2)	2.6-3.7
GLOB (g/dl)	4.1 (0.4)	4.0 (0.3)	4.1 (0.5)	3.9 (0.5)	2.6-4.1
Alb/Glob	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.6-1.4
BT (mg/dl)	1.7 (0.4)	2.1 (0.7)	2.1 (0.7)	2.1 (0.7)	0-3.2
B Dir (mg/dl)	0.2 (0.0)	0.3 (0.2)	0.3 (0.2)	0.3 (0.2)	0-0.4
B Indir (mg/dl)	1.5 (0.4)	1.8 (0.6)	1.8 (0.8)	1.9 (0.8)	0.2-2.0
GLUC (mg/dl)	77.6 (10.1)	88.4 (10.6)	83.9 (11.9)	81.1 (4.7)	62-134
TRIG (mg/dl)	19.4 (4.8)	20.7 (7.7)	22.2 (7.2)	22.3 (9.3)	4-44
CHO (mg/dl)	80.7 (11.3)	77.8 (9.6)	75.5 (11.4)	73.2 (11.6)	75-150
Ca (mg/dl)	11.9 (0.9)	11.6 (1.2)	11.7 (0.5)	11.1 (0.5)	10.2-13.4
P (mg/dl)	2.8 (1.1)*	3.2 (0.9)	3.7 (0.7)*	3.8 (0.8)	1.5-4.7
Na (mg/dl)	133.8 (4.2)*	133.9 (4.0)	131.2 (1.7)*	132.8 (1.6)	128-142
K (mg/dl)	3.9 (0.4)	3.8 (0.4)	3.8 (0.3)	4.0 (0.5)	2.9-4.6
Cl (mg/dl)	101.2 (5.2)	100.6 (4.6)	100.3 (3.3)	99.8 (2.9)	98-109
Mg (mg/dl)	1.6 (0.2)*	1.4 (0.3)*	2.3 (1.0) *	2.0 (0.8)*	1.4-2.3

\* =  $P < 0.05$ **Table 3.** Mean value of lactate serum concentration before and after the first (ST) and the second (ST<sub>TPP</sub>) stress test.

	ST					ST <sub>TPP</sub>				
	T0 Mean (SD)	T1 Mean (SD)	T1+2h Mean (SD)	T1+8h Mean (SD)	T1+18h Mean (SD)	T0 <sub>TPP</sub> Mean (SD)	T1 <sub>TPP</sub> Mean (SD)	T1 <sub>TPP</sub> +2h Mean (SD)	T1 <sub>TPP</sub> +8h Mean (SD)	T1 <sub>TPP</sub> +18h Mean (SD)
Serum lactate concentration	2.1 (4.0)	11.4* (6.1)	2.6 (4.5)	1.6 (3.9)	1.7 (4.1)	2.0 (3.9)	9.8* (5.6)	2.2 (4.2)	1.6 (3.9)	1.5 (3.9)

\* =  $P < 0.05$ 

and T0<sub>TPP</sub> were found for WBC, PDW, AST, BUN, P, Na, and Mg. Differences between T1 and T1<sub>TPP</sub> were found for AST, BUN and Mg. ALP was found to be slightly higher than normal range at T0.

CK was found to be increased between ST and ST<sub>TPP</sub> but the difference was not statistically significant. Lactate dehydrogenase (LDH) was found to be higher than normal ranges in all stage of the study. For lactate concentration differences were found between T1 and T1<sub>TPP</sub> but not for the other sampling times.

## DISCUSSION

The reason for the declines of total WBC count and the increased PDW at rest after administration of TPP are not clear. In both cases, however, the values fell within the normal physiological ranges (24). The lower WBC number may be due to the decrease of the influence of the stress, since the horses were more accustomed to be handled and sampled after the first session. The authors can provide no explanation for the differences in PDW measurement (an index of platelet size uniformity), although change in blood coagula-

tion and platelet indexes has been shown to be induced by exercise in athletic horses (28). Variations of AST, BUN, P, Na and Mg at different stages of the study (mainly when dosed at rest conditions, e.g. T<sub>0</sub> and T<sub>0TPP</sub>) can be considered as normal oscillation within their normal physiological ranges (23, 26). Regarding BUN, the ranges reported in literature are highly variable, ranging from 11–27 mg/dl (23) to 24–48 mg/dl (27). Furthermore, also the values recorded at T<sub>0</sub> and T<sub>1</sub> in the present study can be considered within the normal range for athletic horses, since it is known that exercise can cause about a 60% increase in this value (23). Elevation in alkaline phosphatase (ALP) activity is considered a marker of liver diseases or exhaustion syndrome in horses (23, 29). However, the slight increase of ALP found at T<sub>0</sub> was not associated with clinical or laboratory evidence of liver injuries (e.g. increased AST or GGT) or exhaustion. Since several tissues contain ALP (bone, intestine, placenta, leukocytes) (30) this slight increase could be due to factors not specifically related with the study and not clinically relevant to the aims.

CK was found to be increased at ST<sub>TPP</sub>, although without statistical significance. Increases in CK are used to detect muscular or cardiac damage but none of the horses showed signs of muscular discomfort or cardiac insufficiency during the trial. Values about 500 U/l are considered normal in exercising horses (31) and only large increases of CK value are usually considered of clinical significance (23). Muscle cells are able to release significant quantities of CK without necessarily being damaged (31) and recumbency or shivering can account for slight rise in CK level in serum (22). Moreover, the differences in CK found in the present study, could be due to causes not related to the vitamin administration, such as the short half life in blood (32), the instability when stored (23) or the hemolysis normally induced by the sampling technique (33).

Lactate dehydrogenase (LDH), as other muscular enzymes in horses in training, normally can be found to be increased, which could reflect increases in mitochondrial membrane permeability rather than muscle damage (27).

Serum lactate showed statistically significant lower post-exercise concentrations in horses after treatment with Bicarbossilasi<sup>®</sup>. It is well known that serum lactate accumulation is a consequence of lactate efflux from the muscle during intense exercise, following intramuscular anaerobic production (5). Results of the present study, confirm that supplementation with thiamine improves glucose metabolism and

limits lactate accumulation in muscles, enhancing metabolic pathways of glucose utilization during exercise. The lower serum lactate could be due to the action of TPP at two levels. The first is probably the increase of pyruvate metabolism thus limiting its accumulation and subsequent activation of the anaerobic pathway. The second mechanism could be the increase of decarboxylation of alpha-ketoglutarate to form succinyl-coenzyme A in the Krebs cycle, since TPP acts as a cofactor in this reaction (3).

Previous studies have already shown that the administration of supplemental thiamine to athletes, decreases the levels of serum lactate during exercise, both in humans and horses (18, 21). Current National Research Council recommendation for dietary thiamine is 3 mg/kg of dry matter for maintenance and 5 mg/kg dry matter in working horses (34). Topliff and colleagues demonstrated that horses fed diets with the higher dosage of thiamine (28 mg/Kg dry matter) had the lowest level of blood lactate compared with horse receiving a lower dosage (2 or 4 mg/Kg dry matter) (21). In the present study, TPP was administered intravenously at the dosage of 1 mg/Kg, twice a day for 7 days and a decrease of serum lactate levels was observed. This may make Bicarbossilasi<sup>®</sup> an interesting tool for modulation of energy resources in horses without the need for high dosage and long lasting diet integration. Because horses receive the appropriate amount of thiamine with the diet, and microbial synthesis in the large colon has also been demonstrated, diet integration is not normally required (35). However, TPP could be parenterally administered in horses prone to suffer from lactic acidosis when heavy exercise or intense training regimens are planned. In human athletes, TPP has been demonstrated to be able to decrease blood lactate levels after a single intravenous administration (1 mg/Kg) 24 hours before the stress test (18). Further studies will be necessary to assess if a single injection of TPP before exercise could be effective in preventing lactate excess in muscle and blood in horses. Moreover, different dosages (e.g. higher dosage) should be tested and compared.

Following high intensity exercise, plasma lactate concentrations normally increase, however it starts to decline at a linear rate a few minutes after the end of the activity (36). We demonstrated that Bicarbossilasi<sup>®</sup> may reduce plasma lactate production during and immediately after the exercise but no effect on the lactate rate of decrease during recovery has been demonstrated. This could be explained by the above mentioned specific action of thiamine during exercise:

breakdown of muscle glycogen providing a greater proportion of energy during high intensity exercise (1). Even if TPP administration could account for the lower production of lactic acid, it had no effect on plasma lactate removal from the blood. Furthermore, since training can increase the rate of removal of lactic acid from the blood (37), the standardized training conditions of the horses included in the study may possibly explain the lack of the effect on the disappearance of serum blood lactate.

Administration of thiamine in its active form (TPP, thiamine pyrophosphate) appears to be able to reduce the blood lactate concentration in exercising horses. Since blood lactate concentration reflects muscle accumulation (6), administration of TPP could be a useful tool for preventing or delaying the onset of fatigue.

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#### CONFLICT OF INTEREST

The authors state that no financial or other conflict of interest have biased the work or influenced the results of this study.

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