

## THE EFFECT OF EXERCISE ON PLASMA ACTIVITIES OF LACTATE DEHYDROGENASE AND CREATINE KINASE IN RED-TAILED HAWKS (*Buteo jamaicensis*)

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**ABSTRACT.**—Plasma activities of lactate dehydrogenase (LD) and creatine kinase (CK) have been used as diagnostic indicators of muscle fitness and damage, respectively, in mammals. Activities of these enzymes were measured in three groups of red-tailed hawks (*Buteo jamaicensis*) differing in flight capability (trained, untrained, and disabled) to determine whether their plasma enzyme activities were indicative of muscle fitness and flight training status. After a standardized flight exercise session, blood samples were taken every 24 hr and the plasma assayed for LD and CK activities. In most hawks, LD and CK peaked 24 hr after exercise and gradually declined over the next 48 hr. Both the baseline and peak enzyme activities were affected by the flight status of the hawk. Flight-trained hawks exhibited the lowest basal plasma LD activity of any of the groups and displayed a slight decrease (9%) in LD activity 24 hr after exercise. In contrast, plasma LD activity rose 156% in untrained hawks and 63% in disabled birds 24 hr after exercise; both values were significantly higher than that of the trained group. Plasma CK activity increased significantly over basal levels in both disabled birds (1450%) and untrained birds (731%) 24 hr after exercise, compared to only a moderate increase in CK activity (57%) in trained hawks. These results verify a significant relationship between flight conditioning and plasma activities of LD and CK in hawks that is similar to the mammalian response to training.

**KEY WORDS:** *creatine kinase; exercise; lactate dehydrogenase; plasma enzymes; red-tailed hawk; rehabilitation.*

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El efecto del ejercicio sobre la actividad plasmática de lactato-deshidrogenasa y creatina-kinasa en *Buteo jamaicensis*

**RESUMEN.**—Las actividades plasmáticas de lactato-deshidrogenasa (LD) y creatina-kinasa (CK) han sido usadas en mamíferos como indicadores diagnósticos de la adecuación muscular y daño, respectivamente. Las actividades de estas enzimas fueron medidas en tres grupos de *Buteo jamaicensis*, los que diferían en su capacidad de vuelo (entrenados, no entrenados y incapacitados) con el fin de determinar si sus actividades plasmáticas eran indicativas de adecuación muscular y de su categoría de entrenamiento de vuelo. Después de estandarizar la sesión de ejercicios de vuelo, las muestras de sangre fueron tomadas cada 24 hr y el plasma fue ensayado para medir las actividades de LD y CK. En la mayoría de *B. jamaicensis*, la actividad plasmática de LD y de CK, alcanzaron un máximo 24 hr después del ejercicio y gradualmente declinaba en las siguientes 48 hr. Tanto la actividad basal como la máxima actividad enzimática fue afectada por la categoría de entrenamiento de vuelo de *B. jamaicensis*. Los individuos entrenados mostraron la menor actividad plasmática basal de LD en todos los grupos; y hubo un suave decremento (9%) en la actividad de LD 24 hr después del ejercicio. Al contrario, la actividad plasmática de LD ascendió a un 156% en individuos no entrenados y a un 63% en aves incapacitadas, 24 hr después del ejercicio. Ambos valores fueron significativamente más altos que los obtenidos en el grupo entrenado. La actividad plasmática de CK se incrementó significativamente sobre los niveles basales tanto en aves incapacitadas (1450%) como en aves no entrenadas (731%) 24 hr después del ejercicio, comparadas con un aumento solamente moderado en la actividad de CK (57%) en aves entrenadas. Estos resultados verifican una significativa relación entre el acondicionamiento de vuelo y las actividades plasmáticas de LD y CK en *B. jamaicensis*, los que son similares a los obtenidos en respuesta al entrenamiento en mamíferos.

[Traducción de Ivan Lazo]

Injured raptors that undergo cage confinement during medical treatment experience a marked de-

cline in flight strength and stamina as a result of their confinement and perhaps because of the injury itself. Rehabilitation of injured raptors for release back to the wild involves a program of forced exercise to regain flight coordination and stamina lost during

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convalescence. Recent studies have reported the improvement of aerobic capacity of red-tailed hawks (*Buteo jamaicensis*) with flight training during the rehabilitation period (Chaplin 1989, Chaplin et al. 1989). In these studies, readiness for release was determined by measuring lactic acid production immediately following exercise and rate of its removal. However, this performance test is only an indirect assessment of the development of strength and endurance in muscle fibers.

The activity of specific enzymes in the plasma, such as lactate dehydrogenase (LD) and creatine kinase (CK), have been routinely used as clinical indicators of muscle damage due to exertion and of cardiac and skeletal muscle diseases (Fujii et al. 1983, Apple and Rhodes 1988). In addition, both the degree of histological muscle damage and amount of enzyme release appear to correlate with the intensity or duration of the exercise (Kosano et al. 1986, van der Meulen et al. 1991). Serum LD and CK activities have also been used as indicators of changes in aerobic capacity of muscle during endurance training in mammalian species (Apple and McGue 1983, Holloszy and Coyle 1984, Rogers et al. 1985). Although plasma and tissue enzyme profiles have been reported for a number of raptor species (Gee et al. 1981, Ivins et al. 1985, Hernandez et al. 1990), these data are of limited value because the range for "normal" is so great and the condition, especially flight capacity, of the bird is not known.

In this study, we wanted to determine 1) whether plasma activities of LD and CK accurately reflected the bird's ability to complete a standardized exercise; i.e., indicate flight fitness, and 2) whether the enzyme response to exercise changed as a function of training. To do this, we measured plasma LD and CK activities following exercise in red-tailed hawks undergoing rehabilitation following injury.

#### METHODS

The subjects in this study were admitted to The Raptor Center (TRC) at the University of Minnesota for a variety of injuries. Injuries were treated and subjects began flight rehabilitation according to standard TRC techniques (Martell and Redig 1985, Chaplin 1989, Chaplin et al. 1989). The subjects were divided into three categories based on their flight status; i.e., number of weeks in the flight training program. Flight-trained birds ( $N = 6$ ) were those birds that had been exercised three times per week for more than three weeks and were determined fit to be released. Untrained birds ( $N = 5$ ) were those just beginning their flight training program with 1-3 wk of flight experience. Disabled birds ( $N = 4$ ) were not release candidates and therefore had not been exercised. Because their

injuries impaired the mechanics of their flight, they did not fly well enough to benefit from a flight training program. They were included in this study to represent the starting point of the rehabilitation period and provide an indication of the response of disused muscle to exercise.

An exercise session consisting of eight flights over a standardized distance of 50 m was administered to all subjects, after which blood samples were taken at varying intervals. In three flight-trained hawks, 0.5 ml blood was drawn into heparinized syringes from the basilic vein pre-exercise and at 2.5, 6, 9, 24, 30, 48, and 72 hr postexercise to determine the peak time of plasma enzyme activity. Once the peak was established, blood samples were then taken from all birds in the study only before exercise (time 0) and at 24, 48, and 72 hr postexercise to minimize stress. Blood samples were drawn at 0, 2.5, 6, 9, and 24 hr from two hawks who were not exercised, in order to document whether plasma enzymes changed as a result of handling stress.

Blood was centrifuged after collection and the plasma was stored at 4°C. The plasma was assayed for total enzyme activity using Sigma Chemical Co. Diagnostic Enzyme Kits: LD kit 340-LD and CK kit 45-5. Enzyme assays were performed within the storage time established by the Sigma assay, but most of the assays were performed within 48 hr. Activity was determined by a rate assay from absorbance changes at 340 nm using a Beckman DU-64 spectrophotometer. Plasma LD activity (Sigma units/ml) was converted to International Units/L (IU/L = conversion of 1  $\mu$ M of substrate/min) at 25°C by multiplying by 0.48; plasma CK activity at 25°C was calculated in IU/L by multiplying change in absorbance per 5 min by 5.0 (as per Sigma protocol). Because of small sample sizes in the bird groups, comparisons of enzyme activity between groups were made using a rank sum non-parametric test for two independent samples; significance of differences between means was determined by the Mann-Whitney test (Snedecor and Cochran 1980). Comparisons of enzyme activity between time intervals within the same group of birds were made using a Wilcoxon signed rank test for paired samples (Snedecor and Cochran 1980).

#### RESULTS

Elevated LD activity (>400 IU/L) occurred 0-36 hr postexercise in the three flight-trained hawks used to establish peak plasma enzyme activity (Fig. 1). Peak CK activity (235 IU/L) occurred 24 hr postexercise in these three hawks (Fig. 1). Plasma activity of this enzyme then declined toward the pre-exercise levels throughout the next 24 hr. The enzyme levels of nonexercised birds did not change appreciably during a 24 hr sampling period due to handling (data not shown). However, to minimize the handling and stress of blood sampling on these birds, we elected to take blood samples at 24 hr intervals following exercise for 72-96 hr. The activities of the enzymes at specific time intervals before and after exercise will be referred to hereafter as

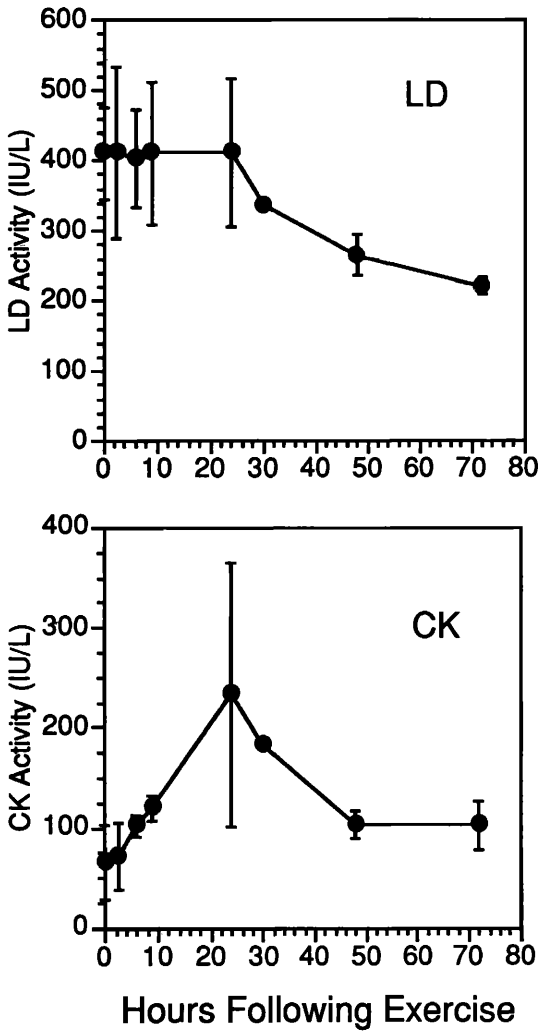


Figure 1. Determination of peak plasma lactate dehydrogenase and creatine kinase enzyme activities (IU/L) by serial blood sampling of three flight-trained red-tailed hawks following an exercise session. Values indicate the mean  $\pm$  1 SE.

CK<sub>0</sub>, LD<sub>0</sub> (preexercise), CK<sub>24</sub>, LD<sub>24</sub> (24 hr postexercise).

Mean LD<sub>0</sub> activity of flight-trained hawks was lower than that of either the untrained or disabled hawks, but not significantly so (Table 1). Mean LD activity of flight-trained hawks actually decreased 9%, 24 hr following exercise (Fig. 2, Table 1). In contrast, exercise caused a marked rise in plasma LD activity in the other two groups of hawks. The

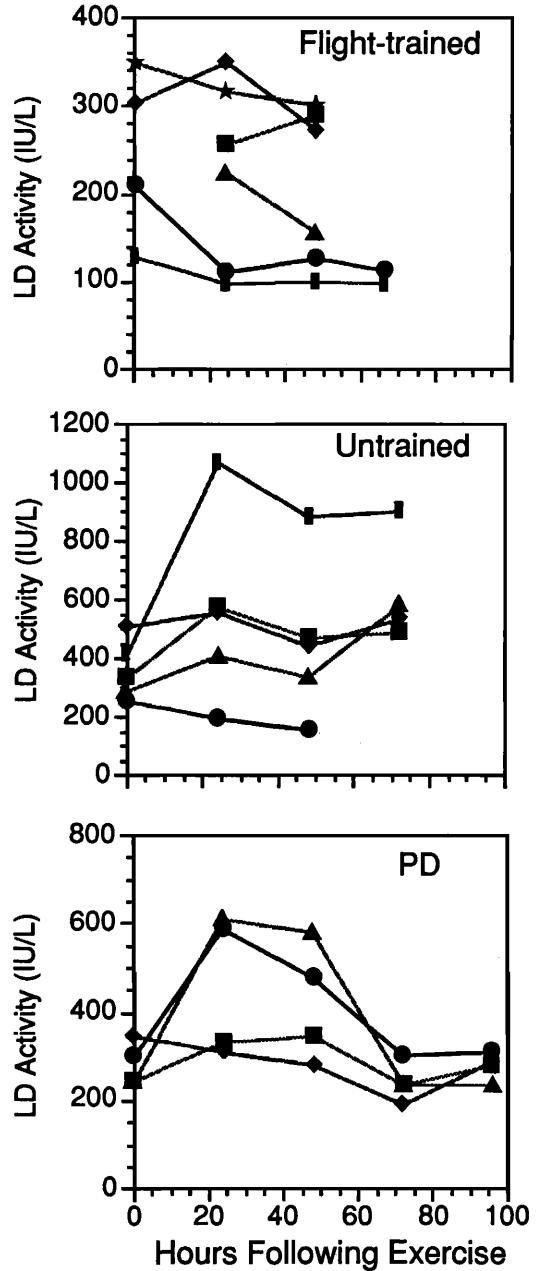


Figure 2. Plasma activity of lactate dehydrogenase (IU/L) in individual red-tailed hawks of each fitness group (flight-trained, untrained, and PD = permanently disabled), as a function of time following exercise.

Table 1. Mean plasma activity of lactate dehydrogenase (IU/L) and (SE) preexercise (time 0) and 24 hr after exercise in three groups of red-tailed hawks.

BIRD CONDITION	<i>N</i>	LD-0 HR (IU/L)	LD-24 HR (IU/L)	<i>P</i> -VALUE <sup>a</sup>
Flight-trained	6	248 (49)	226 (41)	0.06
Untrained	5	360 (46)	561 (123)	0.09
Permanently disabled	4	283 (24)	462 (80)	0.12
Significant groups		None	Trained vs. untrained ( <i>P</i> = 0.02)	
			Trained vs. disabled ( <i>P</i> = 0.01)	

<sup>a</sup> *P*-values indicate differences between enzyme activities preexercise and 24 hr postexercise within one group of birds. Differences between groups at any one time interval are listed under "Significant Groups."

mean plasma LD<sub>24</sub> activity of untrained hawks increased 56% (Table 1) but the increase was not significant due to the large variance in plasma LD<sub>24</sub> activity in this group (Fig. 2). Two of the disabled hawks, whose flight was very labored, also exhibited a marked increase (117%) in plasma LD<sub>24</sub> activity; however, the mean for the disabled group at 24 hr, although higher than LD<sub>0</sub>, was not significantly different from their preexercise level (Table 1). Because LD activity did not peak in flight-trained hawks, but did increase markedly 24 hr after exercise in the other two groups, there were significant differences (*P* < 0.05) between LD<sub>24</sub> activity in trained vs. untrained and trained vs. disabled groups (Table 1).

Mean CK<sub>0</sub> activity of flight-trained hawks was significantly higher (*P* < 0.05) than that of untrained or disabled birds (Table 2); however, there was no significant effect of exercise on plasma CK activity of trained birds. In contrast, CK increased dramatically in almost all untrained and disabled

hawks 24 hr postexercise (Fig. 3). Mean CK<sub>24</sub> activity of untrained birds increased eight-fold in response to exercise, and the mean plasma CK<sub>24</sub> activities in untrained birds was significantly greater (*P* = 0.03) than their respective preexercise levels (Table 2). There was also a dramatic response to exercise in two of the disabled birds (Fig. 3), in which CK<sub>24</sub> activity rose 360% over baseline. Despite the marked enzyme release in some of the untrained and disabled hawks, there were no significant differences between the fitness groups in plasma CK<sub>24</sub> activities because of the variability in response of birds in each fitness group (Table 2).

#### DISCUSSION

LD and CK are cellular enzymes whose activities in the blood following exercise reflect the metabolic and mechanical capacity of the muscle cells in different ways. Dependence upon the LD enzyme dur-

Table 2. Mean plasma activity of creatine kinase (IU/L) and (SE) preexercise (time 0) and 24 hr after exercise in three groups of red-tailed hawks.

BIRD CONDITION	<i>N</i>	CK-0 HR (IU/L)	CK-24 HR (IU/L)	<i>P</i> -VALUE <sup>a</sup>
Flight-trained	6	410 (105)	645 (205)	0.31
Untrained	5	160 (25)	1330 (475)	0.03
Permanently disabled	4	115 (20)	2495 (1175)	0.06
Significant groups		Trained vs. untrained ( <i>P</i> = 0.01)	None	
		Trained vs. disabled ( <i>P</i> = 0.01)		

<sup>a</sup> *P*-values indicate differences between enzyme activities preexercise and 24 hr postexercise within one group of birds. Differences between groups at any one time interval are listed under "Significant Groups."

ing anaerobic respiration results in the accumulation of high levels of lactic acid, which can eventually interfere with muscle contraction. It has been shown that plasma LD activity increases immediately after exercise and peaks about 24 hr postexercise in mammals, and that both resting and postexercise plasma activities of this enzyme decrease with endurance training (Rose et al. 1980, Apple and McGue 1983), as the muscle fibers transform to a more aerobic type of metabolism with decreased dependence on the glycolytic pathway for ATP production.

CK is essential in cellular metabolism in transferring a high-energy phosphate bond from phosphocreatine to ADP, thus forming ATP when energy supplies in the cell are low. Total plasma CK activity increases in proportion to the amount of effort during exercise in mammalian endurance athletes (Rose et al. 1980, Apple and McGue 1983). Thus, plasma CK activity is often used as a diagnostic indicator of muscle damage, which can occur when the muscle mechanical strength and elasticity is exceeded by the exercise effort producing cellular disruption and enzyme leakage (Hortobagyi and Denahann 1989). As muscle cells hypertrophy and neuromuscular coordination improves with training, less cellular disruption occurs, and consequently, plasma CK activities are lower in well-trained athletes (Apple and McGue 1983).

The results of this study suggest that variability in plasma activities of LD and CK are correlated with differences in the flight condition and activity levels of hawks. Flight-trained hawks exhibited the lowest resting levels of LD of any of the groups in this study (Table 1); in fact, their LD activities were at the low end of the range reported for raptor species (Table 3). Lower plasma LD activity in these birds reflects two processes occurring in the muscle during training: 1) decreased reliance on the anaerobic pathway; i.e., decreased production of lactate during exercise; and 2) decreased leakage of enzyme from the muscle. Both processes have been documented in mammalian species (described above), and we suggest that these processes are occurring during flight-training of hawks as well. Plasma LD<sub>24</sub> activities of flight-trained hawks in this study were significantly lower than that of untrained hawks, which suggests that one effect of training on muscles of red-tailed hawks is diminished reliance on anaerobic (lactate) metabolism. In addition, flight-trained hawks exhibited only slight changes in LD and CK enzyme activities as a result of exercise, while the untrained

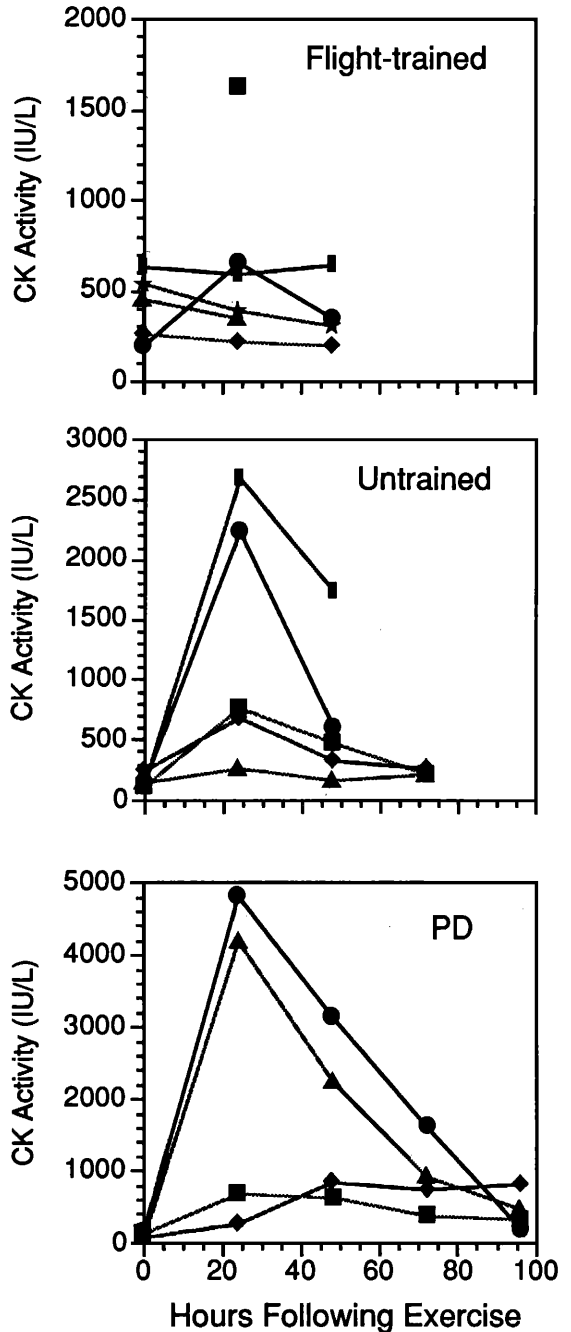


Figure 3. Plasma activity of creatine kinase (IU/L) in individual red-tailed hawks of each fitness group (flight-trained, untrained, and PD = permanently disabled), as a function of time following exercise.

Table 3. Mean (SD) and ranges in lactate dehydrogenase and creatine kinase plasma activities (IU/L) of some raptor species.

SPECIES	N	CK	LD	CONDITION	SOURCE
Common buzzard ( <i>Buteo buteo</i> )	2	393 (188) (119–766)	632 (153) (300–820)	Clinically normal captives	Hernandez et al. (1990)
Peregrine falcon ( <i>Falco peregrinus</i> )	5	ND <sup>a</sup>	870 (224) (575–1117)	??	Gee et al. (1981)
S.A. snail kite ( <i>Rostrhamus sociabilis</i> )	2	ND	524 (74) 471–576	??	Gee et al. (1981)
Andean condor ( <i>Vultur gryphus</i> )	9	ND	274 (56) 192–360	??	Gee et al. (1981)
Bald eagle ( <i>Haliaeetus leucocephalus</i> )	4	ND	250–580	Clinically normal captives	Ivins et al. (1985)
Harris' hawk ( <i>Parabuteo unicinctus</i> )	3	ND	245–400	Clinically normal captives	Ivins et al. (1985)
Golden eagle ( <i>Aquila chrysaetos</i> )	5	ND	320–690	Clinically normal captives	Ivins et al. (1985)
Red-tailed hawk ( <i>Buteo jamaicensis</i> )	4	ND	470–770	Clinically normal captives	Ivins et al. (1985)
Red-tailed hawk	6	410 (257) (197–635)	248 (120) (244–345)	Trained	Present study
Red-tailed hawk	5	160 (56) (115–250)	360 (103) (128–350)	Untrained	Present study
Red-tailed hawk	4	115 (40) (90–375)	283 (48) (252–510)	Permanently disabled	Present study

<sup>a</sup> ND = The study did not determine CK values.

and disabled hawks exhibited marked increases in plasma activities of both enzymes. These data suggest that flight training improved the structural integrity of the muscles, making them less leaky to LD and CK enzymes.

Plasma enzyme activities also reflect the overall activity level of individuals, as evidenced by the fact that flight-trained hawks exhibited the highest resting levels of CK and the relatively immobile disabled hawks the lowest resting levels of CK of the groups in this study. This may have been a result of a higher daily activity level of trained hawks in their flight room. Disabled hawks, because they cannot fly well, tend to be very sedentary, even in large area holding space (Chaplin unpubl. obs.) and would therefore engage in minimal flight muscle activity daily. The fact that there was only a moderate increase in plasma CK activity of flight-trained hawks postexercise suggests that these birds had achieved the muscular strength and elasticity necessary for coordinated flight effort. In contrast, the susceptibility to muscle injury in the other two groups of hawks is suggested by the

significant increases in CK<sub>24</sub> activity and activities elevated above baseline for at least 72 hr. This response is similar to that observed in mammalian species. For example, CK activity peaked 24 hr after a marathon race and stayed elevated for 72 hr in human runners (Rogers et al. 1985).

It has been suggested that the basis for the efflux of certain muscle enzymes, such as CK and LD, into the plasma following exercise is due to both the metabolic and mechanical effects of exercise (Belcastro et al. 1985, Nicholson et al. 1986, respectively). Extensive mechanical stretching, associated with intense levels of muscle activity, has been observed to cause membrane damage and fiber necrosis in wild birds. Pectoral muscle fibers of Canada geese (*Branta canadensis*) showed cellular disruption and lysosomal activity following migration (George et al. 1987). Additionally, the increases in metabolites in the cell produced during exercise may increase the membrane permeability, thereby allowing efflux of enzymes into the circulation (Belcastro et al. 1985). For these reasons, it is likely that vigorous exercise

following a period of reduced activity (e.g., recovery from injury in this study) might produce cellular damage and metabolite concentrations in flight muscles sufficient to cause an efflux of muscle enzymes into plasma.

Thus, the activities of certain muscle enzymes in the plasma appear to reflect both the amount of muscular effort and the degree of muscular fitness for exercise in hawks. Although the exercise effort of hawks in this study could not be regulated or quantified, it was nevertheless obvious that flight-trained birds flew with ease, while disabled hawks struggled to remain airborne during the exercise session and probably sustained the greatest degree of muscle stretching, as indicated by their high CK<sub>24</sub> activities. Similarly, untrained hawks flew well mechanically, but were obviously "out of shape," based on increased respiration observed following exercise and the fact that their LD<sub>24</sub> activities were higher than that of flight-trained birds. If flight capacity and flight performance are, in fact, correlated with LD<sub>24</sub> and CK<sub>24</sub> activities as we have suggested from the results of this study, it would be interesting to determine the plasma activities of these enzymes in migrating birds, to determine whether they might reflect the distance traveled during migration.

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