Effect of Vit. E on Myoglobin Content of Guinea Pig Skeletal Muscle.* (25325)

B. A. SCHOTTELIUS, D. D. SCHOTTELIUS AND A. D. BENDER
Dept. of Physiology, College of Medicine, State University of Iowa, Iowa City

Increase in myoglobin concentration of guinea pig skeletal muscle during early stages of Vit. E-deficiency has been reported(1). This increase was postulated to be due to an increase in anabolic nature of myoglobin metabolism, principally because of the observation by Indovina(2) of an increase in myoglobin turnover in Vit. E-deficient rabbit skeletal muscle. During extended periods on Vit. E-deficient diet, there was a decrease in myoglobin content which Indovina attributed to increase in catabolism over anabolism. Indovina suggested a "normalizing" effect of Vit. E on myoglobin metabolism. A decrease in myoglobin content also has been noted in a) muscles of patients with progressive muscular dystrophy before onset of marked clinical disability(3), b) in calves with muscle degeneration(4). In genetically dystrophic mice diminished myoglobin concentration accompanied by creatinuria has been reported(5). Such observations can be interpreted as revealing either an actual defect in myoglobin metabolism or a passive, secondary loss from damaged muscles. The present study examined quantitatively the myoglobin changes in both gastrocnemius and masseter muscles from guinea pigs carefully selected for age, sex, and weight during prolonged Vit. E-deficient states. Furthermore, we examined the effect of Vit. E-supplementation on previously Vit. E-deficient animals, and correlated, where possible, all observations with other reported criteria of nutritionally induced dystrophy.

Methods. Forty-six English strain male guinea pigs, 30 days of age, were placed on Vit. E-deficient diet(6) for either 21 or 30 days. In the 21 day study, deficient, supplemented (control) and recovery animals were maintained through 14th day as previously described(1). Subsequently, the deficient animals were continued on the deficiency regimen, while the supplemented and recovery animals were given 15 mg of dl-alpha-tocopherol daily until the 21st day. Supplementation of the 30-day control animals with 15 mg of dl-alpha-tocopherol was performed twice weekly. On designated day of sacrifice the gastrocnemius and masseter muscles were removed and analyzed for myoglobin(1), total nitrogen, non-collagen-nitrogen (NCN) and percentage of solids(6). Muscle weight was expressed as percentage of total body weight. Daily creatine excretion was determined on 4 supplemented and 4 deficient animals throughout the 30-day period. Histological sections were made from samples taken randomly and equally from each dietary regimen and duration. Statistical analysis of data was by the small sample t test. A probability of ≤ 0.05 was accepted as revealing a significant difference between the groups.

Results. No statistically significant difference between gastrocnemius or masseter muscles of deficient, supplemented or recovery animals in terms of percentage solids or weights (as percentage of body weight) is evident in the 21 day studies (Table I). Furthermore, no important alteration in water content or weight/body weight ratio is noticeable in muscles from guinea pigs maintained on Vit. E-deficient diet for 30 days. Non-collagen-nitrogen analyses reveal no significant changes except in the 21 day deficient gastrocnemii, which show a decline in NCN concentration. There is a significant diminution of myoglobin concentration (mg/g wet muscle) in deficient muscles at both dietary durations.

In Table II myoglobin concentrations are expressed in mg/g of non-collagen tissue. The percentage difference between control and experimental muscles at 21 and 30 days is not as great in the masseters as in the gastrocnemii; nevertheless, the difference is statistically significant in all instances.

Supplementation of previously deficient animals from 15th to 21st day (recovery study)

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TABLE I. Means and Standard Errors of Myoglobin and Non-Collagen Nitrogen Concentration, Percentage Solids and Muscle Weight as Percentage of Body Weight of Gastrocnemius and Masseter Muscles of Guinea Pigs on Their Respective Diets and Durations.

Dietary regimen and duration		No. of cases	Myoglobin (mg/g wet muscle)	Non-collagen N (mg/g wet muscle)	% solids (dry wt/wet wt × 100)	Muscle wt/body wt × 100
			Gastrocnemius			
21 day	Def.	13	.964 ±.054*	23.37 ± .92*	23.64 ±.24	.497 ±.016
	Suppl.	10	1.373 ±.085	26.29 ± .69	24.03 ±.45	.501 ±.018
	Rec.	10	1.240 ±.089†	26.10 ± .69†	23.68 ±.33	.499 ±.011
30 day	Def.	7	1.141 ±.057*	23.75 ± .34	23.65 ±.45	.478 ±.026
	Suppl.	6	1.519 ±.073	25.31 ± .64	23.88 ±.49	.503 ±.024
			Masseter			
21 day	Def.	13	3.128 ±.085*	24.31 ± .54	23.89 ±.23	.718 ±.026
	Suppl.	10	3.740 ±.167	26.73 ±1.08	23.58 ±.42	.715 ±.024
	Rec.	10	3.665 ±.223†	25.43 ± .54	24.02 ±.30	.735 ±.020
30 day	Def.	7	3.136 ±.049*	24.01 ± .56	23.89 ±.35	.727 ±.033
	Suppl.	6	3.926 ±.197	25.16 ± .43	24.16 ±.36	.704 ±.021

* Probability of difference between deficient and supplemented groups $\leq .01$.
† " " " " " " recovery groups $\leq .01$.

prevents depletion of myoglobin and maintains the NCN level. No elevation in urinary creatine appears even after 30 days on the deficient diet. The histological picture of gastrocnemius muscles in the 21 day deficient group is one of mild or incipient degenerative changes; this state is not evident in either the recovery or supplemented groups. At 30 days, the pathology in deficient gastrocnemii approaches that described by West and Mason (7) as a sub-lethal type of injury. In contrast, masseter muscles from both 21 and 30 day deficient groups exhibit only rare and very mild cytological alterations.

TABLE II. Mean and Standard Error, Percentage Difference and Probability of Difference in Myoglobin Concentration per Gram of Non-Collagen Tissue.

Dietary regimen and duration		Myoglobin (mg/g NCN tissue)	% difference (Def. *vs* suppl.)	Probability
		Gastrocnemius		
15 day	Def.	1.844 ±.125		
	Suppl.	1.508 ±.055	+22.3	.030
21 day	Def.	1.291 ±.078		
	Suppl.	1.687 ±.100	−23.5	.004
	Rec.	1.586 ±.110		
30 day	Def.	1.520 ±.089		
	Suppl.	1.912 ±.083	−20.8	.008
		Masseter		
15 day	Def.	4.725 ±.206		
	Suppl.	3.738 ±.248	+26.3	.034
21 day	Def.	4.114 ±.140		
	Suppl.	4.640 ±.290	−11.4	.040
	Rec.	4.605 ±.295		
30 day	Def.	4.087 ±.118		
	Suppl.	4.901 ±.204	−16.4	.006

Discussion. Short-term lack of Vit. E in the diet produces a progressive and marked increase in myoglobin concentration (1). Extension of the deficiency duration precipitates an equally marked decline in the concentration. These changes in muscle pigment occur in both red (masseter) and pale (gastrocnemius) muscles before appearance of severe cytological lesions or of elevated creatine excretion. It is appropriate, however, to consider 2 sources of error in estimating the myoglobin status. First, there is the possibility of differences in dilution resulting from inequalities in muscle water content. This aspect has been remarked upon by Anthony *et al.*(8). Second, an analogous situation might arise if there were significant changes in the non-collagen/collagen tissue ratio.

Although there was no evidence of a significant change in water content, in view of the observed decline in NCN of the 21 day deficient gastrocnemii, all myoglobin data were recalculated and expressed (Table II) as mg/g non-collagen tissue. The re-evaluation yielded an identical conclusion: *i.e.*, that depression in myoglobin concentration was real and of reasonably great magnitude. Fifteen-day values were included to emphasize how suddenly the concentration was reversed, from 22% greater to 23% less than control levels in the case of gastrocnemii. It is apparent

that the percentage decline was considerably smaller in the red muscle than in the pale muscle. This finding is in accord with the statement by Aloisi(9) that the pale muscles of the guinea pig are more sensitive to Vit. E-deficiency than are red muscles. Moreover, Goettsch and Pappenheimer(10) have reported that the masseter is the last muscle in the guinea pig to reveal any pathological lesion of nutritional dystrophy.

Increase in myoglobin during early stages of Vit. E-deficiency is probably due to an anabolic error. The subsequent decrease in myoglobin concentration after 21 days is subject to one or both of 2 interpretations: a) catabolism exceeds anabolism; or b) passive loss from muscle occurs. Certainly the decline in myoglobin concentration is greater than can be accounted for on the basis of degenerative changes observed in histological sections. On the other hand, even the presence of rare and mild cytological lesions might imply subtle and extensive membrane permeability changes not recognizable in histological sections. That an abnormal muscle permeability occurs in mice with hereditary muscular dystrophy has been suggested by the studies of Zierler(11).

Supplementation of previously Vit. E-deficient animals, as performed in 21 day recovery studies, prevents the abrupt and marked reversal in myoglobin concentration observed in the 21 day deficient animals. Although there is an actual decline in pigment concentration in recovery guinea pigs, the magnitude of concentration at 21 days is comparable to that recorded for control animals. The response of recovery animals to Vit. E-supplementation attests to the immediacy and potency of the vitamin in "normalization" of myoglobin metabolism.

Summary. A marked decline in myoglobin concentration has been demonstrated in gastrocnemius and masseter muscles of guinea pigs maintained longer than 15 days on a Vit. E-deficient diet. Supplementation with Vit. E after 14 days on a deficient diet reduces the magnitude of concentration change and, indeed, stabilizes pigment concentration at control levels. No significant difference between control and experimental animals in regard to water content, muscle weight or body weight was evident from 21 and 30 day dietary regimen studies. Non-collagen nitrogen was significantly depleted only in the gastrocnemii of the 21 day deficient animals.

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