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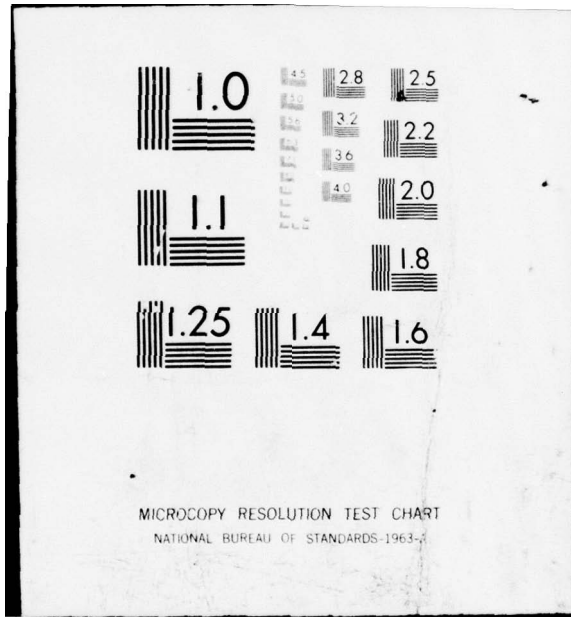
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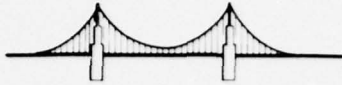
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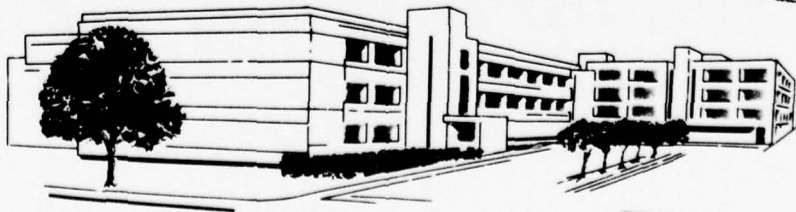
RIBOFLAVIN DEPLETION AND WORK CAPACITY IN HUMANS

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ABSTRACT

Six moderately active young adults received a riboflavin deficient diet (< 0.07 mg/day) for 56 days. Submaximal work performance was measured at three levels on the treadmill — 4, 7, and 10% grade — all at 3.4 mph. Physiological parameters, e.g., heart rates, pulmonary ventilation, oxygen uptakes in ml/kg/min taken during the depletion-repletion period were all essentially unchanged from pre-depletion values. The study demonstrated that performance was not significantly changed during riboflavin depletion as compared with pre-depletion value.

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PREFACE

C. Frank Consolazio, 1913-1976, one of the world's foremost nutritional physiologists, began his scientific career in 1929 as a laboratory technician at the Harvard Fatigue Laboratory in Boston. In 1947, he joined the Federal service as a physiologist. He served at Army medical nutrition laboratories in Chicago and in Denver where he became the Chief, Bioenergetics Division. He continued in this capacity at Letterman Army Institute of Research, San Francisco, where he was an active member of the staff at the time of his death. Mr. Consolazio authored more than 200 scientific publications and participated in approximately 100 human nutrition-related field studies. His contributions to science and, in particular, to military nutrition are a lasting memorial to a man who was not only an outstanding scientist, but also a beloved friend, and an inspiration to those who knew him and were privileged to work with him.

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INTRODUCTION

The relationship between riboflavin depletion and work capacity in humans has not been thoroughly investigated. Most of the earlier studies were designed to evaluate multi-vitamin (B-complex) depletion to establish the daily minimal requirements for preventing the occurrence of the vitamin deficiency syndromes (1-6). A majority of these studies showed a deterioration of physical work capacity.

In one controlled study, Keys et al. (7) fed a restricted riboflavin diet for 148 days (0.31 mg/1000 kcal consumed) and observed no physiological or clinical impairment. These intakes were below the NRC daily riboflavin allowance of 0.55 mg/1000 kcal consumed, which is adequate to meet the known needs of healthy persons who perform moderate physical activities (8).

However, many factors may influence riboflavin excretion, e.g., protein and energy intakes, metabolic body size, and absorption of an oral vitamin load (8,9).

The depletion study was designed to evaluate enzymatic measurements (erythrocyte glutathione reductase) of riboflavin status in man during riboflavin depletion and subsequent repletion (10). The study had not

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1. Egana, E. et al. *Am J Physiol* 137:731, 1942
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 3. Consolazio, C.F. et al. *Metabolism* 5:259, 1956
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 7. Keys, A. et al. *J Nutr* 27:165, 1944
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been designed to produce a clinical riboflavin deficiency, nor were any changes of clinical riboflavin deficiencies observed during the short period of the study. The two protein levels were studied to evaluate whether any relationship existed between protein metabolism and riboflavin requirements (5,11). Since limited information was available concerning the riboflavin status of persons in relation to work capacity and psychological behavior, these areas were also investigated. Two studies have been published, the enzymatic measurement of riboflavin status in man (10), and the behavioral effects while consuming a restricted riboflavin intake (12). This report is the last one in this series where work capacity was evaluated during riboflavin depletion.

METHODS AND MATERIALS

Six healthy men, between 19 and 24 years of age, weighing between 70-83 kg served as subjects. The subjects were housed on our Metabolic Ward where they received and consumed a liquid diet deficient in riboflavin for 56 days. Half of the subjects received 120 g of protein/day and others received 60 mg of protein/day. Additional subjects were not available to have independent concurrent controls.

The formulated liquid diets contained all of the essential vitamins and minerals that exceeded the NRC allowances, with the exception of riboflavin being maintained at the < 0.07 mg/day level during the depletion period (11). Physical activity was limited to maintain body weight equilibrium.

The study was conducted in three phases: a pre-depletion period of 14 days, a depletion period of 56 days, and a repletion period of 10 days (Table 1). The subjects were generally sedentary except for the weekly performance test on the treadmill. The environmental conditions during performance testing were maintained at $22^{\circ} \pm 1.2^{\circ}\text{C}$ with relative humidity ranging between 30-40%. Energy metabolism measurements were made at rest and while walking on a treadmill at 3.4 miles/hour at a 4, 7, and 10% grade for 15 minutes each. The work levels were continuous and the grades were increased (e.g., 4-7%, etc.) for a total work time of 45 minutes.

Measurements at each work level included heart, respiration, and pulmonary ventilation rates, gas temperature, barometric pressure, and analysis of expired gases (oxygen and carbon dioxide). Oxygen uptakes

11. Rivlin, R.S. New Eng J Med 283:463, 1970

12. Sterner, R.T., and W.R. Price. Am J Clin Nutr 26:150, 1973

were calculated from these parameters (13). The last 5 minutes of each steady state work level were combined and used for comparisons. At the end of the 45-minute submaximal work period, the grade was again increased to 15%, and those individuals who were able to complete this 15-minute test then worked to exhaustion by increasing the grade an additional one percent/min. This additional test was to evaluate the effects of the deficient riboflavin diet on endurance. At the end of the rehabilitation phase (end of study), a maximal modified Balke test (14) was made to evaluate work capacity for the two levels of protein intake.

Statistical comparisons were made among protein group means and across performance levels. An analysis of variance, on the repeated measures design, was made comparing the pre-depletion, depletion, and repletion phases. Neuman-Keuls multiple comparisons tests were also made with significance evaluated at the $P < 0.05$ level (15).

RESULTS

The average body weight change from the end of pre-depletion to the end of the depletion period was 1.2 kg (for 56 days).

Initial comparisons did not reveal any significant effects of the two protein levels on parameters for work performance so both protein groups were combined for further evaluation. The mean heart rates/min for rest and three work levels are presented in Table 2. No significant differences were observed at rest or any of the three work levels throughout the entire study. Respiration rates and respiratory exchange ratios (RQ) remained essentially unchanged (from pre-depletion values) during the depletion and repletion periods. Variations within each work level were small.

Pulmonary ventilation (\dot{V}_E BTPS in liter/min) are summarized in Table 3. The values for the entire depletion and repletion phases were not significantly different from the pre-depletion values at all work levels. However, ventilation rates showed a tendency to decrease at all metabolic levels of work as the deficiency period progressed.

Oxygen uptakes expressed in liters/min, taken during the depletion and repletion phases, were not significantly different from pre-depletion values at all work levels. In general, the highest values occurred during the pre-depletion measurements. Resting oxygen uptakes (liters/min)

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13. Nelson, R.A. et al. Report No. 318, USAMRNL, 1968
 14. Balke, B. Report No. 1. USAF School Aviat Med, 1952
 15. Winer, B.J. Statistical principles in experimental design (second Edition). McGraw-Hill, 1971

were not significantly different from the pre-depletion values. The lowest resting value was observed on the last day of depletion, while the lowest work values occurred one week earlier. A similar pattern occurred when oxygen uptakes were expressed in ml/kg/min (Table 4). The decrease in oxygen uptakes with time suggested a training effect.

The total work times (in minutes) attained during the endurance performance tests were not significantly different from the pre-depletion values. The average endurance times ranged between 51.8 to 55.3 min, with the longest work time occurring during day 3 of depletion.

The maximal work capacity test was avoided until the end of the study, since there is some evidence that anaerobic work may cause a significant decrease in urinary riboflavin excretion (10). The data suggest that both protein groups were in a comparable state of physical fitness at this time (Table 5). Maximal heart rates, maximal \dot{V}_{O_2} , ml/kg/min, \dot{V}_E liter/min, total work times, and the sum of the recovery heart rates were slightly but not significantly higher in the low protein group. However, the ROs were significantly higher.

DISCUSSION

In spite of the severity of the riboflavin depletion of body stores demonstrated by the activity coefficients of erythrocyte glutathione reductase (10) and the significant psychological changes that were stress related (12), physical work performance was not impaired in this study. Riboflavin Intakes of < 0.07 mg/day were considerably less than intakes of 0.31 mg/1000 kcal consumed in the Keys et al. study (7); however, both studies were in agreement.

As with any essential nutrient, there is no doubt that an inadequate supply of riboflavin would in time limit work performance and other functions. This was the situation in the Taiwan study (3) where energy metabolism was significantly increased. The Taiwan study (3) entailed a long-term B-complex vitamin deficiency where the clinical signs of vitamin deficiencies were prominent, and under conditions in which the men were on a high daily energy expenditure regimen.

CONCLUSIONS

Physiological work parameters at the end of 56 days were not impaired during three levels of submaximal work as compared to the pre-riboflavin-depletion values.

RECOMMENDATIONS. None.

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A P P E N D I X

Table 1. Riboflavin intake*

Phase		mg riboflavin/day
Pre-depletion	14 days	1.5
Depletion	56 days	< 0.07
Repletion	7 days	0.5
Repletion	1 day	3.5
Repletion	2 days	15.0

*The formulated diet contained approximately 3000 kcal to maintain body weight and included all the essential nutrients that exceeded the NRC allowances with the exception of riboflavin (11).

Table 2. Mean heart rates/min*

Phase	Sitting Rest		Treadmill Walk 3.4 mph					
	Mean	SD	4% grade		7% grade		10% grade	
			Mean	SD	Mean	SD	Mean	SD
Pre-depletion Days								
3	89	9	127	16	142	24	155	16
10	85	9	126	9	140	13	158	16
Restriction Days								
3	83	11	126	10	140	15	156	18
10	80	17	120	14	138	18	156	17
17	80	9	128	10	143	14	157	16
24	83	9	127	12	138	18	158	17
31	80	11	122	12	138	14	156	16
38	84	9	127	9	140	11	156	11
45	81	13	126	12	138	15	157	15
53	82	3	127	15	138	10	153	12
Rehabilitation Days								
3	85	12	127	11	139	13	159	14
10	82	10	129	15	142	16	160	18

*No significant differences between control and any of the phases.

Table 3. Pulmonary ventilation, liter/min BTPS*.

Phase	Sitting Rest Mean SD		Treadmill Walk 3.4 mph					
			4% grade		7% grade		10% grade	
			Mean	SD	Mean	SD	Mean	SD
Pre-depletion Day								
3	12.8	2.0	42.3	4.8	52.9	2.8	66.8	9.0
10	12.2	2.0	41.8	4.4	51.6	5.6	65.0	9.6
Restriction Day								
3	12.0	2.0	41.0	5.2	52.0	6.2	65.9	10.7
10	12.0	2.0	40.0	5.8	50.6	6.4	64.4	10.3
17	11.4	1.6	39.2	4.4	49.2	5.7	63.5	9.7
24	12.7	3.2	40.5	4.6	51.2	5.9	64.9	10.5
31	11.1	1.8	38.5	6.3	48.6	8.4	63.8	10.8
38	11.6	1.6	39.9	4.5	48.8	5.9	62.6	9.4
45	11.2	3.0	38.7	5.5	47.7	6.2	62.3	8.6
53	10.9	2.1	39.4	5.5	48.8	5.0	63.4	8.0
Rehabilitation Day								
3	12.0	3.1	40.2	4.2	49.0	5.8	65.6	9.1
10	12.0	2.8	39.2	4.5	50.2	6.0	64.6	10.6

*No significant differences between controls and any other phases.

BTPS - At body temperature, ambient pressure and saturated with water vapor.

Table 4. Oxygen uptakes, ml/kg/min*.

Phase	Treadmill walk 3.4 mph							
	Sitting Rest		4% grade		7% grade		10% grade	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre-depletion Day								
3	4.8	0.6	19.0	0.8	24.0	1.0	28.5	1.4
10	4.7	0.6	18.9	0.6	23.3	1.2	28.1	1.5
Restriction Day								
3	4.5	0.3	18.7	1.2	23.3	1.4	28.4	1.6
10	4.5	0.6	18.5	1.3	23.4	1.8	27.8	2.0
17	4.2	0.5	18.3	1.0	22.5	1.1	28.1	1.4
24	4.8	0.8	18.7	0.8	23.5	0.8	28.7	1.3
31	4.2	0.4	18.2	2.1	22.5	2.6	28.0	2.5
38	4.5	0.4	18.7	1.3	22.7	1.5	28.4	1.8
45	4.3	0.9	18.0	1.8	22.1	1.8	27.6	1.8
53	4.0	0.6	18.2	2.3	22.4	1.4	27.9	1.7
Rehabilitation Day								
3	4.0	0.5	18.6	1.2	23.0	1.4	28.5	1.4
10	4.6	0.8	18.6	1.1	23.4	1.3	28.7	1.2

*No significant differences between controls and any of the phases.

Table 5. Maximal work capacity.

Parameter	Protein intake/day			
	120 g		60 g	
	Mean	SD	Mean	SD
Heart rate/min	187	5	192	5
Respiration rate/min	46	3	43	11
\dot{V}_E (liter/min BTPS)	118.4	7.9	123.5	5.7
RQ	1.00	0.01	1.11*	.04
Oxygen uptake (liter/min)	3.23	0.16	3.20	0.36
Oxygen uptake (ml/kg/min)	43.1	3.5	41.7	4.4
Work time, minutes	21.6	2.2	21.8	4.1
Sum of recovery heart rates	363	84	403	14

*3 men/group. This test was done after rehabilitation. No significant differences were observed between groups (except RQs).

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