The effect of exercise on the riboflavin status of adult men

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(*Received 29 July 1991 – Accepted 20 May 1992*)

Six sedentary to moderately active men with biochemical signs of riboflavin deficiency were studied under metabolic ward conditions to examine the effects of physical activity on riboflavin status. All participants were subjected to additional exercise (EXER) for an 18 d period between two maintenance (M1 and M2) periods (16 and 13 d respectively) of habitual physical activity. Energy balance and riboflavin intake were maintained throughout the study. Riboflavin status, as judged by a significant reduction in erythrocyte glutathione reductase (*EC* 1.6.4.2) activation coefficient (EGR-AC), improved on changing from home (1·53 (SD 0·14)) to period M1 (1·36 (SD 0·21)) diets. The exercise period, however, resulted in a significant deterioration in riboflavin status (1·57 (SD 0·31)) which persisted in the subsequent period M2 (1·54 (SD 0·15)). There was a concomitant fall in the urinary excretion of riboflavin only in the EXER period, when results were expressed as a percentage of the dietary intake of riboflavin. These results suggest an increased demand for the vitamin for selective biochemical functions during exercise. However, the energy cost of walking (treadmill 4 km/h), 50 W and 100 W work-loads (bicycle ergometer) as well as delta mechanical efficiency (DME) did not change during the three metabolic periods. The urinary excretion of riboflavin was inversely related to DME (r −0·49; $P < 0·05$) and directly correlated with haemoglobin levels (r 0·63; $P < 0·005$). The present study suggests that riboflavin status further deteriorates during a short period of increased physical activity in individuals whose riboflavin status is marginal.

Riboflavin: Glutathione reductase: Physical activity: Mechanical efficiency

Studies confined to clinical cases of malnutrition fail to assess the situation in its entirety since they only address the proverbial tip of the iceberg. Investigations on subclinical or biochemical malnutrition, which are major problems in India, are important to rule out any functional consequences on the health and productivity of the population. In India riboflavin deficiency is very common (National Nutrition Monitoring Bureau, 1980), being possibly modulated by dietary as well as non-dietary factors (infection, physical activity etc.; Belko *et al.* 1983; Bamji *et al.* 1987). The basis of the present study was the observed seasonal variations in the riboflavin status of rural Indian women, as assessed by erythrocyte glutathione reductase (NADPH glutathione oxidoreductase; *EC* 1.6.4.2; EGR) activation coefficients (EGR-AC) (National Institute of Nutrition, 1988). Although a majority of women had marked biochemical riboflavin deficiency throughout the year, there was a significant deterioration in riboflavin status during the monsoon season compared with the summer or winter seasons. This change could not be attributed to changes in diet or morbidity. Since during the rains there was augmented physical activity

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due to active farming, a probable association between riboflavin status and physical activity was inferred.

In laboratory-based studies Belko *et al.* (1983, 1984, 1985) observed that increased levels of physical activity result in a lowering of riboflavin status. Hence, the present study was designed to obtain further evidence for the proposed hypothesis that physical activity influences riboflavin status. The energy cost of physical activity as well as delta mechanical efficiency (DME) were monitored for evidence of any functional change during this period.

MATERIALS AND METHODS

Six apparently healthy adult males were recruited from amongst the unskilled workers of the Institute. Subjects were between 27 and 47 years of age and had body weights between 43·5 and 86·3 kg, with a body mass index (BMI) ranging between 17·2 and 30·2 kg/m^2. Selected candidates had no clinical signs or symptoms of systemic disease or vitamin deficiencies, were weight stable for 6 months before the study and gave no history of chronic medication. Prestudy measurements included medical history, clinical and anthropometric evaluation, estimation of haemoglobin and riboflavin status, habitual physical activity patterns and the assessment of the ability to complete a supervised test run on a bicycle ergometer at a 100 W load for 20 min.

Protocol

All subjects resided in the metabolic ward of the Institute for the duration of the study. The 47 d study was divided into three periods: an initial maintenance period (M1) of 16 d, an exercise period (EXER) of 18 d and a final maintenance period (M2) of 13 d. Measurements of metabolic, haematological and biochemical variables were made at the end of each period.

Experimental diets

During periods M1 and M2 all subjects, regardless of body weight, were fed on a diet of approximately 10·5 MJ (2500 kcal)/d with a riboflavin intake of 0·42 mg/4·2 MJ (1000 kcal) per d per person. Although the body weight of one of the subjects was much higher than that of the other five, his energy intake at home was comparable with those of the other subjects and, hence, a single maintenance diet was given to all the subjects regardless of body weight. During the exercise period, an additional 1·25 MJ (300 kcal)/d per person was provided to compensate for the increased energy cost of the exercise (Table 2). This increase in energy was such that the nutrient composition (% of energy and riboflavin intake (mg/4·2 MJ per d)) was similar to that of periods M1 and M2 and was achieved by reducing the intake of wheat (flour) and supplementing the diet with rice, one egg and sugar. Riboflavin intakes were initially computed from standard Indian food tables (Gopalan *et al.* 1987). However, a marginally higher intake of riboflavin during EXER (0·46 mg/4·2 MJ per d) compared with that during periods M1 and M2 was observed when duplicate portions of a random day's diet were analysed, in each period, for riboflavin content (Morell & Slater, 1964; Table 1). Bulk food items were purchased on one occasion, while fresh vegetables were purchased daily. All food was cooked by one person after standardizing cooking procedures. Raw food was individually portioned before being cooked. Canned synthetic diets were omitted. The food items served were chosen to represent a typical low- to middle-income South Indian diet to ensure subject compliance.

Physical activity

The subjects were requested to maintain their usual levels of physical activity and, hence, were permitted to attend their daily work routine. During the exercise period all subjects

Table 1. *Composition of dietary intake (g/d) during the study*

Metabolic periods*...	M1 and M2	EXER
Wheat (flour)	150	100
Rice	300	380
Sugar	50	80
Egg	—	50
Red gram†	50	50
Milk (ml/d)	150	150
Green leafy vegetables	50	50
Other vegetables	150	150
Banana	70	70
Oil	20	20
Condiments and spices	12	12
Energy‡		
MJ/d	10·34	11·63
kcal/d	2472	2781
Protein‡ (g/d)	61·4	67·5
Riboflavin§ (mg/d)	1·04	1·28
Iron‡ (mg/d)	40·2	38·0

* For details, see p. 542.
† *Cajanus cajan.*
‡ Computed from standard Indian food table (Gopalan *et al.* 1987).
§ Actually measured in duplicate samples of food.

performed supervised exercises on a bicycle ergometer (100 W load) as well as on a treadmill (4 km/h level) in two separate sessions, each of 30 min, daily for 6 d/week. While treadmill walking was started at the prescribed speed, bicycling was started at lower loads and gradually brought up to the desired load and duration over the first 3 d. Such a programme did not, on any occasion, warrant a premature stoppage in any subject. Total energy expenditure (24 h; TEE) was obtained from physical activity records by interviewing each subject (recall method) on two week days and one weekend day in each period. The activity records were coded into nine work intensity zones and the time-period spent by each person under each work zone was obtained. Energy expenditure within each work zone was obtained by multiplying the time spent with the energy cost of that zone (adjusted for body weight). These values for nine zones for a 60 kg person were obtained from the literature (Bouchard *et al.* 1983). Some more typical Indian activities were also put into these work intensity zones (Satyanarayana *et al.* 1988). Adding up the energy expenditures from all nine zones, total energy expenditure (TEE) for 24 h or 1440 min was obtained for each subject.

Energy expenditure and body composition measurements

Anthropometric measurements were made using standard techniques (Weiner & Lourie, 1969). Body fat was calculated from the sum of four skinfolds using equations of Durnin & Womersley (1974). Energy costs of walking and cycling at 50 W and 100 W loads were determined by standard procedures with the help of validated techniques involving Douglas bags, dry gas meter (Model DTM-325; Singer, American Meter Division, USA) and paramagnetic O_2 (Servomex, type OA-272; Taylor, Sybron Corp., Herts.) and infra red CO_2 analyser (Medical gas analyzer LB-2; Beckman Instruments, Schiller Park, IL, USA) as described previously (Durnin *et al.* 1988). Basal metabolic rates (BMR) were measured in the baseline period under standard conditions using the same instrumental set up. Due to malfunctioning of the O_2 analyser during EXER period, all subsequent

measurements of O_2 were made on a Scholander Micrometer gas analyser (Toshniwal Instruments and Engineering Co., New Delhi, India). The performance of the Scholander Micrometer gas analyser was checked daily against a previously analysed standard gas mixture. The variations in O_2 determinations for this period were within 0·63%. Work done was converted into energy equivalent (1 kcal = 427 kpm/min). The mechanical efficiency of subjects for accomplishing 306 kpm/min work, from 306 kpm/min (or 50 W) to 612 kpm/min (or 100 W), was obtained by using general principles adopted previously by Astrand (1960). The mechanical efficiency (ME) formula of Astrand (1960) was modified to obtain delta mechanical efficiency (DME):

$$\text{ME} = \frac{\text{Mechanical work performed (pkm/min)}}{427 \times (\text{work (kcal/min)} - \text{Basal (kcal/min)})} \times 100,$$

$$\text{DME} = \frac{\text{Mechanical work } (612 - 306 = 306 \text{ kpm/min})}{427 \times (\text{kcal at } 612 \text{ kpm/min} - \text{kcal at } 306 \text{ kpm/min})} \times 100.$$

The DME value is expected to be neutral to body size, as the body mass influence will be seen in both the denominator and the numerator. This procedure was previously used to report values for DME status of rural Hyderabad subjects in comparison with Swedish subjects (Satyanarayana *et al.* 1989). Physical activity levels (PAL) were calculated as TEE: 24 h BMR (James *et al.* 1988).

Urine and blood analysis

Timed 24 h urine samples were collected over the last two consecutive days of each period of the study. Portions were stored at −4° and subsequently analysed for creatinine (Oser, 1965) and riboflavin content (Morell & Slater, 1964).

Blood samples were collected into heparinized tubes at the end of each period and haemoglobin estimated by the cyanmethaemoglobin method. Saline (9 g NaCl/l)-washed erythrocytes (after separation of leukocytes and platelets) were stored at −20°. Riboflavin status was assessed by measuring erythrocyte glutathione reductase activity and its *in vitro* stimulation with FAD (EGR-AC) (Bayoumi & Rosalki, 1976).

Statistical analysis

All results are expressed as means and standard deviations and the baseline measurements compared with period M1 by a paired t test at the 5% significance level. All values for the three metabolic periods were analysed using a two-way analysis of variance (ANOVA) without replicates for differences between subjects and between metabolic periods. When F ratios were significant at the 5% level, Newman–Keuls multiple comparison test was used to locate the differences (Winer, 1971). Since one subject was febrile at the end of period M2, no metabolic or biochemical measurements were made. Hence, the harmonic mean of observations was used to assess the critical difference in the Newman–Keuls procedure (Winer, 1971). Correlation coefficients between measured variables were calculated for the pooled values over the three periods, for seventeen pairs of observations.

Ethical approval

The study was approved by the Ethical Committee of the Institute and all subjects gave written, informed consent.

RESULTS

The baseline measurements (Table 2) indicated that all subjects were normally nourished based on their BMI and PAL according to recent criteria for chronic energy deficiency

Table 2. *Pre-study baseline measurements in six adult men*
(Mean values and standard deviations)

	Mean	SD	Range
Age (years)	35	7·3	27–47
Weight (kg)	55·90	15·6	43·6–86·3
Fat-free mass (kg)	44·6	8·10	37·0–60·1
Body mass index (kg/m^2)	20·9	4·77	17·2–30·2
Basal metabolic rate			
kJ/kg FFM per d	133·82	15·22	111·24–151·38
kcal/kg FFM per d	32·0	3·64	26·6–36·2
Total energy expenditure			
MJ/d	10·96	4·36	8·33–19·61
kcal/d	2620	1042	1993–4689
Physical activity level	1·83	0·55	1·50–2·94
Treadmill walking			
kJ/min	15·22	4·60	10·58–20·87
kcal/min	3·64	1·10	2·53–4·99
Bicycle ergometer			
50: W kJ/min	15·89	0·84	14·68 17·02
kcal/min	3·80	0·20	3·51 4·07
100 W: kJ/min	27·60	1·46	26·68 29·98
kcal/min	6·60	0·35	6·38–7·17
DME	25·7	2·69	21·0–27·7
Haemoglobin (g/l)	150	21·2	125–176
Riboflavin status			
EGR basal activity*	181	60·7	98–260
EGR stimulated activity*	272	87·1	170–417
EGR-AC	1·53	0·139	1·35–1·73

EGR, erythrocyte glutathione reductase (*EC* 1.6.4.2); EGR-AC, EGR activation coefficient.
* μmol NADPH oxidized/h per g haemoglobin.

(James *et al.* 1988). One subject, however, was obese (BMI > 30 kg/m^2). Haemoglobin status was good and DME was within the range of well-nourished Indians (Satyanarayana *et al.* 1989). Two subjects were marginally normal in riboflavin status (EGR-AC 1·2–1·4) while the remainder were deficient, having EGR-AC values > 1·4.

At the end of period M1 there was a significant fall in EGR-AC and rise in EGR basal activity compared with the baseline values (paired *t* test; $P < 0{\cdot}05$; Tables 2 and 3). There were no changes in any other variables with subjects maintaining body weight and composition. The TEE in this period (Table 4) was slightly lower than the baseline values (Table 2).

The ANOVA over the three metabolic periods revealed significant differences within subjects between periods in EGR-AC (F 5·58; df 2·9; $P < 0{\cdot}05$). The EGR-AC was significantly higher in the EXER period as well as period M2 compared with period M1 (Table 3). The changes in EGR basal and EGR stimulated activity, however, were not statistically significant. Despite the marginally higher riboflavin intake during the EXER period the urinary excretion of the vitamin as a percentage of intake was significantly lower in this period compared with periods M1 and M2 (F 10·26; df 2·9; $P < 0{\cdot}05$).

There were no within-subject differences in body weight, fat-free mass and TEE during the study. Other measured variables such as haemoglobin, cost of walking, cycling at 50 W or 100 W and DME also showed no differences over the three periods. Significant differences between subjects were, however, evident for all variables studied. Correlation analysis when done with pooled values for all the subjects from all the periods showed that

Table 3. *Effect of physical activity on riboflavin status during the study of six adult men*

(Mean values and standard deviations)

Metabolic periods...	M1			EXER			M2†		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Haemoglobin (g/l)	152·0	25·4	118·0–184·0	143·0	19·8	119·0–168·0	142·0	19·7	118·0–163·0
Urinary riboflavin									
μg/24 h	273	95·2	160–425	232	68·6	135–315	231	78·8	114–319
μg/g creatinine	240	76·3	143–311	220	71·5	141–299	223	62·5	162–299
% of intake	26·2	9·2	15·4–40·9	18·1*	5·4	10·5–24·5	22·3	7·6	11·0–30·7
EGR basal activity†	236	60·4	146–324	258	60·6	158–313	233	64·8	180–307
EGR stimulated activity‡	316	76·9	234–411	389	35·8	335–428	356	89·7	273–483
EGR-AC	1·36	0·21	1·35–1·73	1·57†	0·31	1·28–2·12	1·54†	0·15	1·35–1·76

EGR, erythrocyte glutathione reductase (*EC* 1.6.4.2); EGR-AC, EGR activation coefficient.

Mean values were significantly different from those in period M1 (overall significance determined by a two-way ANOVA, while multiple comparisons between periods were made by the Newman–Keuls test): $*P < 0{\cdot}05$.

† n 5 during period M2; for details, see p. 544.

‡ μmol NADPH oxidized/h per g haemoglobin.

Table 4. *Effects of physical activity on body composition and energy expenditure during the study of six adult men*

(Mean values and standard deviations; overall significance was assessed by a two-way ANOVA; there were no significant differences between metabolic periods)

Metabolic periods...	M1			EXER			M2*		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Wt (kg)	56·1	15·5	46·4–86·3	56·8	16·3	44·1–88·3	56·2	16·6	43·6–88·5
FFM (kg)	44·5	7·89	37·5–59·2	44·2	7·09	36·7–57·2	43·9	7·57	36·5–58·0
Total energy expenditure									
MJ/d	10·33	3·26	7·73–16·64	11·01	3·24	8·14–17·19	10·64	4·18	7·88–18·72
kcal/d	2471	778·4	1848–3979	2633	775·5	1946–4111	2545	1000	1885–4477
kJ/kg FFM per d	228·8	30·61	200·7–281·0	245·5	30·5	221·6–300·7	236·7	47·7	197·0–322·8
kcal/kg FFM per d	54·7	7·32	48·0–67·2	58·7	7·3	53·0–71·9	56·6	11·4	47·1–77·2
Treadmill walking									
kJ/min	13·76	3·39	10·87–19·74	13·05	3·39	10·29–19·66	13·76	4·39	9·62–19·66
kcal/min	3·29	0·81	2·60–4·72	3·12	0·81	2·46–4·70	3·29	1·05	2·30–4·70
kJ/kg wt per h	14·80	1·09	13·72–16·18	13·84	0·79	13·34–15·43	13·51	1·09	12·63–14·18
kcal/kg wt per h	3·54	0·26	3·28–3·87	3·31	0·19	3·19–3·69	3·23	0·26	3·02–3·39
Bicycle ergometer									
50 W: kJ/min	16·06	1·13	15·22–18·23	14·80	1·21	13·72–16·94	15·77	0·92	14·72–17·15
kcal/min	3·84	0·27	3·64–4·36	3·54	0·29	3·28–4·05	3·77	0·22	3·52–4·10
100 W: kJ/min	27·22	2·09	25·13–30·57	26·14	2·38	23·54–27·73	26·26	2·13	24·59–29·86
kcal/min	6·51	0·50	6·01–7·31	6·25	0·57	5·63–6·63	6·28	0·51	5·88–7·14
DME (%)	26·9	3·67	20·3–30·2	27·1	5·64	20·3–30·2	28·4	3·53	23·1–32·1

FFM, fat-free mass; DME, delta mechanical efficiency.

* *n* 5; for details, see p. 544.

DME was negatively correlated with urinary excretion of riboflavin (r 0·485; $P < 0·05$), while haemoglobin levels were positively correlated with urinary excretion of the vitamin (r 0·634; $P < 0·005$).

DISCUSSION

The study was designed to examine the effects of increasing physical activity on the riboflavin status of adult men. Estimation of TEE in free-living subjects is a difficult task, but this procedure becomes easier when the subjects are confined to the metabolic ward. The Canadian method of Bouchard *et al.* (1983) was adapted to the Indian situation. In this approach subjects gave an account of their social use of time to the interviewing investigator. Subjects with minimum educational background with time consciousness can account for social utilization of time to the nearest 15 min. Thus, the 24 h day (or 1440 min) was divided into nine intensity zones. This method is expected to reflect major variations in the energy expenditure patterns of subjects in the different periods and is a generally adopted technique in literate groups to quantify energy expenditure. While in the original procedure described by Bouchard *et al.* (1983) the record of physical activity is maintained by the volunteer, in the modified method of Satyanarayana *et al.* (1988) the information is elicited by interviewing the candidates by a trained person.

The baseline TEE of the group was about 10·9 MJ (2600 kcal)/d and PAL values indicated that the group was engaged in moderate levels of physical activity (Table 2).

Traditional diets of low-income-group Indians are known to be deficient in riboflavin. This was evident in the high EGR-AC values before the study. The diet served from the metabolic kitchen was a typical low- to middle-income South Indian diet and provided only 0·42 mg of the vitamin/4·2 MJ per d. Nevertheless, it was perhaps superior to the home diet and resulted in significant improvement in riboflavin status as seen by the significant fall in EGR-AC and rise in EGR basal activity at the end of period M1.

All subjects maintained body weight and composition over the entire duration of the study. Despite increased physical activity resulting from the supervised exercise equivalent to 1·25 MJ (300 kcal), TEE was not significantly different between the three periods. This may be due to the normally large variability in habitual activity within and between subjects. It would, therefore, appear that the observed changes in EGR-AC may be due to differences in the type of physical activity as well as some increase in total activity. The significant rise in EGR-AC during the EXER period was not due to a dietary deficiency of riboflavin since EGR basal was unchanged from that of period M1. In fact, both EGR basal and EGR stimulated tended to increase, the relatively greater rise in the latter resulted in higher EGR-AC during exercise. There were no clinical signs of deficiency during this period.

Similar observations were made on women subjected to an exercise regimen while on a riboflavin intake of 1·16 mg/4·2 MJ per d. However, on a lower intake of 0·96 mg/4·2 MJ per d the increase in EGR-AC with exercise was due to a fall in EGR basal activity (Belko *et al.* 1985). In addition, Belko *et al.* (1983) have shown that sedentary women required 0·96 mg/4·2 MJ per d to normalize riboflavin status (i.e. EGR-AC < 1·25), while during an exercise programme the same women required 1·1 mg/4·2 MJ per d. The observations that EGR stimulation tends to increase during exercise would suggest that exercise results in an increase in EGR apoenzyme, either due to greater synthesis or due to greater stability. This would imply greater demand for FAD during exercise.

It has been reported that during regular exercise riboflavin accumulates in muscle (Turkki & Lepisto Hunter, 1985) and FAD-dependent enzymes such as succinate dehydrogenase (*EC* 1.3.99.1) (Mole *et al.* 1971; Gollnick *et al.* 1973) increase. This may represent part of the mechanism signalling the shift from carbohydrate to fat utilization during exercise training. The change in riboflavin status observed in the present study and

in those of Belko *et al.* (1983, 1984, 1985) may, hence, be a metabolic consequence of short-term regular exercise and tentatively suggests an increased demand for the vitamin during such periods. The concomitant fall in the excretion of riboflavin seen in the present study and in those of Tucker *et al.* (1960) and Belko *et al.* (1984, 1985) would support these conclusions and denotes an attempt to conserve the vitamin during periods of increased activity.

Failure to normalize the biochemical variables in period M2 despite discontinuation of the exercise may be a carry-over effect, as well as delayed detraining.

Interestingly, over the entire duration of the study the urinary excretion of riboflavin was directly correlated with haemoglobin levels and inversely correlated with DME. Of the six subjects, three subjects had consistently higher DME values and consistently lower excretion of riboflavin in urine (< 200 μg/g creatinine). This trend was true in all three periods and the correlation coefficient value for the seventeen pairs (six subjects; three periods (one subject missed measurements in period M2)) was significant. However, the predictive power of this observation is limited ($y = 601 - 12{\cdot}77x$).

Riboflavin is known to influence Fe absorption and reduced flavins are important in Fe mobilization (Zaman & Verwilghen, 1977; Adelekan & Thurnham, 1986; Powers *et al.* 1988). However, a direct effect of exercise *per se* on haemoglobin levels (pseudodilution anaemia) cannot be ruled out. The observation that the excretion of riboflavin was also negatively related to muscular efficiency might suggest that the previously mentioned variables are perhaps the acute metabolic effects of the exercise programme.

Despite the changes in riboflavin status in the present study the cost of physical activity, i.e. walking and cycling at 50 W and 100 W loads, did not exhibit any change. DME or muscular efficiency also did not differ between study periods. This may be due to the short duration of the study. Supplementation with riboflavin did not improve the work performance of children (Powers *et al.* 1987; Prasad *et al.* 1990) or athletes (Tremblay *et al.* 1984; Weight *et al.* 1988). Also, individuals on a restricted intake did not demonstrate any deficits in physical work performance (Keys *et al.* 1944). However, in the present study the highest work-load employed was only 100 W or 612 km/min per min. Physically active men can handle three to four times this work-load as the maximal work-load. Clearly the work-load selected in the present study was to elicit work performance (or energy conversion efficiency of man) at sub-maximal work-load levels. No attempt was made in the present study to estimate maximal O_2 uptake (or V_{O_2max}) of the six subjects under study. Any change in the performance which may manifest at higher exercise intensity cannot be discussed from the results of the present study. Efficiency of subjects measured at sub-maximal levels did not show measurable variation with changes in the riboflavin status.

Such results imply either that riboflavin is unconnected with aerobic work at sub-maximal levels of work or that physiological function is maintained over a wide range of EGR-AC values. Recent studies suggest that current guidelines for the interpretation of EGR-AC may be too stringent (Campbell *et al.* 1990; Prasad *et al.* 1993). While the present study does not permit any comment regarding the redefinition of interpretary guidelines, it does point to the need for further work.

In conclusion, while the present study has demonstrated a deterioration in riboflavin status (as judged by the glutathione reductase test) during an exercise regimen, concomitant changes in the cost of work or muscular efficiency were absent, suggesting a conservation of physical function.

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Printed in Great Britain