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MYOGLOBIN CONTENT AND ENZYMATIC ACTIVITY
OF HUMAN SKELETAL MUSCLE-THEIR RELATION
WITH THE PROCESS OF ADAPTATION TO
HIGH ALTITUDE

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FOREWORD

This report was prepared in the Institute of Andean Biology and the Department of Pathological Physiology, Faculty of Medicine, Lima, Peru, by:

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ABSTRACT

Quantitative determinations of myoglobin were made in the sartorius muscle of healthy human subjects, native of sea-level and high-altitude areas. The specific activity of the reduced form of diphosphopyridine nucleotide oxidase (DPNH-oxidase), DPNH- and TPNH-cytochrome C reductases, transhydrogenase, and isocitric and lactic dehydrogenases were also examined. A significantly higher myoglobin concentration was found in the muscle of the high-altitude natives as compared with sea-level residents. The enzyme systems DPNH-oxidase, TPNH-cytochrome C reductase, and transhydrogenase similarly showed a significantly higher activity in altitude residents. It was concluded that the respiratory capacity of the muscle was apparently higher in natives living at high altitude than in those living at sea level. The enhanced enzymatic activity was probably related to the higher pigment content of the skeletal muscle. Results on myoglobin determinations in several other muscles from certain sea-level patients are discussed.

This technical documentary report has been reviewed and is approved.



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MYOGLOBIN CONTENT AND ENZYMATIC ACTIVITY OF HUMAN SKELETAL MUSCLE— THEIR RELATION WITH THE PROCESS OF ADAPTATION TO HIGH ALTITUDE

1. INTRODUCTION

Studies on enzymatic processes during adaptation to high altitude have been done chiefly in small animals, and, in most instances, this is the only way to approach the problem. It should be recognized that the process of acclimatization to a new environment need not be similar in all species. In fact, it may be different, and the more evolved the organism the more delicate the process may be. Therefore, results obtained in small animals must be cautiously applied to man.

In the present investigation the activity of some oxidative enzymes and the concentration of the respiratory pigment myoglobin have been examined in muscle biopsies obtained from sea-level and high-altitude natives.

2. MATERIAL AND METHODS

Experimental subjects

A group of 9 healthy, young natives of Cerro de Pasco, Peru, 4,400 meters above sea level, was studied. None of them had ever been at sea level nor lived continuously at an altitude lower than that of Cerro de Pasco for more than a year before this experiment. All of them were employees of the hospital or of a local hotel. In Lima, 50 meters above sea level, a total of 39 natives of the coast was studied. Nine of the 39 were healthy young men, similar to the altitude group in activity, racial characteristics, and age. The other 30 subjects from sea level were surgical patients in a local hospital.

Biopsies were taken from the sartorius muscles of the healthy sea-level and high-

altitude subjects under identical operative conditions. From the patients at sea level, other cross-striated muscles were also obtained. In all cases, a piece of muscle approximately 700 mg. was excised between clamps. After blotting the excess blood, the sample was divided into two portions approximately equal. Each portion was accurately weighed in a Roller-Smith balance and placed in the interior of a conveniently fitted Potter-Elvehjem glass homogenizer. The latter was immediately corked and chilled in an ice bath until the assays were performed. The time that elapsed between the excision and the assays was never longer than 1 hour.

Preparation of homogenates and cell fractions

The sample destined for enzymatic assays was homogenized in 9 volumes of cold isotonic sucrose (0.25 M), as previously described (1). A small aliquot of the homogenate was taken aside for DPNH-oxidase activity determinations and the remaining homogenate was centrifuged in a model PR-1 International refrigerated centrifuge at $900 \times g$ for 15 minutes. The resulting supernatant was collected and the residue suspended in 4 volumes of cold isotonic sucrose, homogenized once more for a few seconds and centrifuged again at $700 \times g$ for 10 minutes. The supernatant obtained was mixed with the one obtained in the first centrifugation and the residue discarded. The pooled supernatants were centrifuged at $24,000 \times g$ for 15 minutes. The supernatant of this last centrifugation was collected and mixed with isotonic sucrose so that 10 ml. were equivalent to 1 gm. of fresh tissue. The residue was resuspended in cold isotonic sucrose of 1 ml. of sucrose solution

per gram equivalent of fresh tissue. This fraction will henceforth be referred to as the mitochondrial fraction, although no ultramicroscopic evidence of their structure has been obtained.

Pigment determinations

The method was essentially similar to that described previously (2). However, since there is not general agreement in the literature concerning the extinction coefficient of hemoglobin at 568 $m\mu$ (2, 5), a relative value for this coefficient was experimentally determined. To this end, human whole blood was prepared in the same manner as tissue extracts for pigment determinations (2), and the spectrum was read from 530 to 590 $m\mu$. By taking 14.7×10^3 as the reference extinction coefficient of hemoglobin at 538 $m\mu$, the following relative extinction coefficients at 568, 574, and 582 $m\mu$ were found: 14.7×10^3 , 12.45×10^3 , and 6.7×10^3 , respectively. The uncorrected extinction coefficients of myoglobin at 538, 568, 574, and 582 $m\mu$ were taken as 14.7×10^3 , 11.8×10^3 , 12.45×10^3 , and 11.8×10^3 , respectively.

Since the results of the extinction coefficient of hemoglobin at 538 and 568 $m\mu$ were equal, the Poel's equation for calculating the concentration of myoglobin was accordingly simplified. The extinction coefficient for the combined metacyano derivative at 540 $m\mu$ was 11.5×10^3 .

Tissue hemoglobin content was calculated by subtracting the myoglobin content from the total pigment content. The latter represented an average of three values: one obtained as the metacyano derivative at 540 $m\mu$, and the others as carbon monoxide compounds at two isobestic points, located in the combined hemoglobin-myoglobin spectrum at 538 and 574 $m\mu$.

Nitrogen determinations

Nitrogen determinations were carried out by the micro-Kjeldahl procedure whenever sufficient material was available.

Assay procedure and reaction mixtures for the determination of enzymatic activity

Enzymatic activity was determined spectrophotometrically in a model DU Beckman spectrophotometer at 28° C.

DPNH-oxidase system

This system was assayed in whole homogenates as previously described for skeletal muscle of guinea pig (1).

DPNH- AND TPNH-cytochrome C reductases and pyridine nucleotide transhydrogenase activities

These activities were determined by using methods and reaction mixtures previously described (6), except for the amount of tissue used. The reaction mixtures were as follows: for DPNH-cytochrome C reductase, 5 mg. equivalents of both mitochondrial and supernatant fractions (0.1 ml. of a 1/2 dilution in isotonic sucrose of the mitochondrial suspension and 0.05 ml. of supernatant); for TPNH-cytochrome C reductase, 0.2 ml. of the mitochondrial fraction (200 mg. equivalents); and for transhydrogenase 0.1 ml. (100 mg. equivalents) of the same mitochondrial fraction.

TPN-isocitric dehydrogenase

TPN-isocitric dehydrogenase was assayed by a method which was essentially that of Ochoa (7), based on the spectrophotometric determination of TPN reduction. The reaction mixture used was as follows: 1 ml. of potassium phosphate buffer 0.1 M at pH 7.4; 0.1 ml. of dl-isocitrate (potassium salt) 0.05 M, 0.1 ml. of $MgCl_2$ 0.06 M, 0.1 ml. of KCN 0.01 M, 0.1 ml. of TPN 5×10^{-3} M; 0.3 ml. of supernatant fraction (30 mg. equivalents); or 0.1 ml. (100 mg. equivalents) of mitochondrial fraction when sufficient material was available, and when enough water was available to make as much as 3 ml. Cyanide was used to prevent reoxidation of TPNH through the respiratory chain. Similar results were obtained when glycylglycine buffer and manganese as activator (7) were used instead of phosphate buffer and magnesium (8).

TABLE I
Nitrogen determinations in the sartorius muscle of healthy native subjects from sea level and high altitude

	Whole tissue (mg. of N/gm. fresh tissue)	Supernatant fraction (S ₁) (mg. of N/gm. equivalent of S ₁)
Sea level	31.23 ± 1.998 (8)	6.40 ± 0.836 (6)
High altitude	31.15 ± 1.489 (6)	6.39 ± 0.277 (9)

Values given are means ± standard deviations. Figure in parenthesis indicates number of subjects.

Lactic dehydrogenase

Lactic dehydrogenase activity was measured in supernatant fraction only since the activity in the mitochondrial fraction was negligible. The method used was the one described by Kornberg (8, p. 441). The reaction medium was as follows: 1 ml. of phosphate buffer 0.1 M at pH 7.4, 0.1 ml. of potassium pyruvate 0.05 M, 0.1 ml. of DPNH 5×10^{-3} M, 0.25 ml. of a 1/50 dilution in isotonic sucrose (0.5 mg. equivalents) of supernatant fraction, and enough water to make up to 3 ml. Cyanide did not change the rate of the reaction.

Definition of units and specific activity

One unit of enzyme was arbitrarily defined as that amount which causes a change in the optical density (E_{240} or E_{330}) of 0.001 per minute under the conditions previously established. Specific activity was expressed in units per 10 mg. of fresh tissue or 10-mg. equivalents of the mitochondrial and supernatant fractions.

3. RESULTS

Normal values of myoglobin at sea level and at high altitude

People living at high altitude seemed to have a higher concentration of myoglobin than those living at sea level (table II). The difference between the mean myoglobin values in the two groups was significant ($P < .02$) whether the results were expressed per gram of fresh tissue or per gram of nitrogen, since

the nitrogen content of the muscle was practically the same in both groups (table I).

The hemoglobin content of the muscle sample

The hemoglobin content of the muscle sample was significantly higher (3.65 mg. per gram of fresh tissue, $P < .02$) in the high-altitude group than in the sea-level group (table II). This means a 60% greater content of hemoglobin in the tissue sample from people living at high altitude than in tissue from sea-level residents. It should be stressed, however, that under the conditions of the present investigation, the hemoglobin content of the muscle sample does not strictly represent the hemoglobin content of the muscle, since unavoidable changes in the blood content of the sample occur during clamping and excision.

The myoglobin content of different muscles

The results of pigment determinations of various cross-striated groups of muscles from sea-level patients are shown in table III. A distinction has been made between patients with apparently normal physical activity (group I) and those who were confined to bed for long periods of time (group II). In the first group, thoracic, abdominal, and limb muscles were studied (table III).

The data revealed a considerable difference in the myoglobin content of the three groups of muscles. The thoracic muscle, serratus

TABLE II
*Sartorius muscle and blood pigments of healthy native subjects
from sea level and from high altitude*

Subject	Age (years)	Hematocrit	Blood hemoglobin (gm.%)	Myoglobin (mg./gm. fresh tissue)	Hemoglobin content of the tissue (mg./gm. fresh tissue)
Sea level					
1	29	44.9	15.1	5.34	11.22
2	30	43.0	14.7	4.98	6.84
3	29	46.0	15.5	5.63	4.92
4	29	42.0	14.1	6.22	3.96
5	27	42.5	13.6	7.27	2.58
6	37	42.0	13.8	5.69	4.32
7	24	42.0	13.8	6.21	8.15
8	25	39.0	13.2	6.92	6.39
9	29	48.0	15.9	6.40	5.00
Mean $\pm$ S.D.	28.8	43.3 $\pm$ 2.50	14.41 $\pm$ 0.88	6.07 $\pm$ 0.70	5.93 $\pm$ 2.44
High altitude					
1	21	50.2	16.2	6.39	11.32
2	25	54.0	17.6	7.20	13.23
3	20	51.0	16.5	6.68	13.72
4	25	52.1	16.7	8.30	8.02
5	28	50.0	16.2	5.86	9.40
6	20	52.0	17.0	7.03	5.13
7	32	50.0	16.3	7.85	8.95
8	24	58.3	19.0	6.45	5.25
9	24	54.0	17.8	7.50	11.24
Mean $\pm$ S.D.	24.3	52.4 $\pm$ 2.55	17.03 $\pm$ 0.89	7.03 $\pm$ 0.73	9.58 $\pm$ 2.94
P*		<.001	<.001	<.02	<.02

*Based on t-test significance of differences between means.

major, seemed to have the highest concentration of myoglobin; more determinations would be necessary, however, to establish a statistically valid comparison with the myoglobin content of other groups of muscles. Abdominal and limb muscles, on the other hand, were clearly different in respect to their myoglobin content. The difference of 1 mg. of myoglobin per gram of fresh tissue in favor of the limb muscles was statistically significant ($P < .01$). The myoglobin concentration in the rectus abdominis

muscle from patients confined to bed (group II, table III) appeared to be lower than from patients with apparently normal physical activity, although the difference was not significant.

Enzymatic activity of the sartorius muscle in healthy subjects from sea level and from high altitude

The enzymatic study of the muscle was carried out in whole tissue as well as in cell-free preparations—i.e., the so-called mitochondrial

TABLE III
*Myoglobin and hemoglobin determinations in
surgical patients from sea level*

Subject	Age (years)	Surgical operation	Myoglobin (mg./gm. fresh tissue)	Hemoglobin content of the tissue (mg./gm. fresh tissue)	Blood hemoglobin (gm./100 cc.)
Group I					
<i>Thoracic muscles*</i>					
13	29	Hydatidostomy	6.56	3.85	10.00
24	30	Hydatidostomy	8.33	3.27	14.80
28	38	Hydatidostomy	5.27	4.98	12.70
Mean			6.72	4.03	12.50
<i>Abdominal muscles†</i>					
17	15	Appendectomy	5.15	9.45	13.90
34	20	Appendectomy	5.51	11.14	13.60
8	22	Cholecystectomy	4.46	5.72	
19	27	Hernioplasty	5.62	6.87	12.60
25	28	Appendectomy	5.51	4.09	11.80
4	39	Appendectomy	5.90	8.30	16.60
20	50	Cholecystectomy	4.93	10.35	10.40
26	52	Hernioplasty	3.52	21.48	9.90
15	54	Cholecystectomy	5.75	10.65	14.40
23	60	Hernioplasty	4.69	15.61	14.70
5	62	Hernioplasty	5.34	22.71	10.50
18	70	Hernioplasty	4.70	10.12	11.00
Mean ± S.D.			5.09 ± 0.65	11.40	12.67 ± 2.05
<i>Limb muscles‡ § ¶</i>					
2	1	Fracture—femur	6.33	5.13	
27	79	Fracture—femur	6.33	7.27	
31	28	Fracture—femur	5.86	5.60	12.50
14	59	Amputation	5.08	2.05	11.45
29	29	Saphenectomy	6.40	11.03	15.90
32	50	Saphenectomy	5.40	8.93	14.90
35	53	Saphenectomy	6.04	5.86	14.10
30	20	Saphenectomy	6.22	7.18	
33	25	Fracture—femur	7.61	0.04	14.70
Mean ± S.D.			6.14 ± 0.67	6.63 ± 2.51	13.92
Group II					
<i>Abdominal muscles</i>					
10	57	Gastric neoplasty	4.92	8.88	11.00
11	72	Intestinal neoplasty	4.92	14.08	11.16
16	52	Gastric neoplasty	3.28	10.62	9.00
7	19	Gastric ulcer	3.75	17.35	13.50
9	28	Gastric ulcer	5.63	9.67	10.39
22	48	Duodenal ulcer	4.70	5.30	10.39
Mean ± S.D.			4.53 ± 0.79	10.99	10.91

*Serratus superior.

†Rectus abdominis.

‡Vastus externus.

§Sartorius.

¶Peroneus.

TABLE IV

The specific activity of some enzymes and enzyme systems in the sartorius muscle of native subjects from sea level and high altitude

Enzyme	Sea level	High altitude	P
Whole homogenate			
DPNH-oxidase system	41.38 ± 2.79 (8)	52.09 ± 11.86 (9)	<.05
Mitochondrial fraction			
DPNH-cytochrome C reductase	57.86 ± 16.37 (9)	65.66 ± 10.15 (7)	NS
TPNH-cytochrome C reductase	0.83 ± 0.29 (8)	1.48 ± 0.39 (7)	<.01
Transhydrogenase	1.46 ± 0.61 (8)	2.59 ± 0.97 (9)	<.05
Supernatant fraction (S₁)			
DPNH-cytochrome C reductase	97.85 ± 36.55 (8)	104.10 ± 16.71 (7)	NS
Isocitric dehydrogenase	10.99 ± 2.78 (8)	14.78 ± 4.65 (9)	NS
Lactic dehydrogenase	1358.14 ± 79.90 (7)	1321.10 ± 143.10 (9)	NS

Values given are means ± standard deviations. Figures in parentheses indicate number of subjects.

and supernatant fractions. The results are presented in table IV. Although the variability in these data (see the S. D.) was probably due in part to the normal biologic variation between subjects, it was, presumably, largely due to limitations in the fractionating technic in obtaining always identical yields of cell fractions.

The activity of the DPNH-oxidase system (in whole homogenate) was greater in high-altitude residents than in sea-level residents (table IV). The difference between the two groups was statistically significant ($P < .05$). The specific activity of this enzyme system in the thigh muscle (rectus anterior) of guinea pigs from sea level and high altitude was 104.3 ± 3.75 and 136.8 ± 5.20 , respectively (1).

As to the cell fractions, all the oxidative enzymes and enzyme systems studied showed invariably a higher activity in the altitude than in the sea-level group. Significant differences, however, were found only in the mitochondrial fraction for transhydrogenase and TPNH-

cytochrome C reductase ($P < .05$ and $P < .01$, respectively). In the supernatant fraction, the microsomal DPNH-cytochrome C reductase enzyme system and the isocitric dehydrogenase enzyme did not show significant differences in activity between sea-level and altitude residents. The glycolytic enzyme lactic dehydrogenase, on the other hand, exhibited identical activity in both groups.

Not shown in the table are the results of determinations on the activity of mitochondrial TPN-isocitric dehydrogenase from the sartorius muscle of 9 healthy subjects from high altitude and 4 patients from sea level (subjects 29, 30, 32, and 35 from table III). The mean specific activities were 1.1 units and 1.3 units for the high-altitude group and the sea-level group, respectively.

4. DISCUSSION

There is no general agreement regarding the effects of high-altitude exposure on the myoglobin content of skeletal muscle. Hurtado

et al. (9) showed that there was a considerably higher level of this pigment in several muscles of dogs native to high altitude. Tappan and Reynafarje (2) more recently have shown that the pigment content of various groups of skeletal muscles was generally higher in guinea pigs from high altitude than in those from sea level. Moreover, when sea-level animals were taken to high altitude, the myoglobin content of their muscles was significantly increased. Anthony et al. (10) have shown that there is a considerable increase in the myoglobin content of the skeletal muscles of rats exposed continuously to a simulated altitude of 18,000 feet. According to these authors, however, the increment is more apparent than real because the change could be accounted for by a concomitant loss of body weight, which they attributed to dehydration. In the same report a true and absolute increment of the pigment in the heart muscle was also observed (10). Poel (3), on the other hand, reported values of myoglobin that were less in rats exposed intermittently to a simulated altitude of 25,000 feet than the values obtained for sea-level controls. In the heart muscle, nevertheless, he also found a real increment of the pigment in response to the hypoxic stimulus. These conflicting results may be due to the variety of experimental conditions used by different workers.

From the results presented in this paper it appears that the myoglobin concentration in the sartorius muscle of high-altitude natives was higher than in the same muscle of sea-level natives. These results agree with those of Hurtado et al. (9) and Tappan and Reynafarje (2). The physiologic significance of these findings, however, remains to be further elucidated.

The higher concentration of myoglobin seen in the altitude native is probably real since the nitrogen content of the muscle (table I), the lean body mass (11), and the body water content (11, 12) were the same in both high-altitude and sea-level subjects.

The pigment apparently is not uniformly distributed in all the muscles of the body

(table III); it seems, however, that muscles from the same area have very similar concentrations. Thus, for example, the thigh muscles, vastus externus and sartorius (table III, group I), have 5.90 and 6.01 mg. of myoglobin per gram of fresh tissue, respectively.

No significant difference was found between myoglobin concentration in patients resting in bed for long periods of time (table III, group II) and in those of different ages (table III, group I), or in those of apparently normal physical activity (table III, group I). Changes in myoglobin concentration, on the other hand, were apparently followed by similar changes in the content of blood hemoglobin. Thus, the order of magnitude of the rise and fall of these two pigments was always about the same. The rise, consequent to altitude exposure, was 18% and 16% for hemoglobin and myoglobin, respectively (table II). Their fall, after continued bed rest, was 13.9% and 11.0% for hemoglobin and myoglobin, respectively (table III, group II).

In connection with the hemoglobin content of the muscle sample, the results obtained under the conditions of the present investigation were only suggestive of a greater capillarity in the muscle of the high-altitude native. Further and more accurate determinations are required to clarify this important point.

Enzymatic activity of the human muscle

The present data revealed that the oxidative activity of the muscle adapted to high altitude was generally higher than at sea level. The enzymatic change occurred apparently preferentially in the mitochondrial fraction of the cell, suggesting that the respiratory enzymes are essentially involved in the process of acclimatization to high altitudes. Although an increased activity of the DPNH-oxidase system and transhydrogenase had been shown before in guinea pigs of high altitude (1), the very large increment in the activity of the TPNH-cytochrome C reductase, as observed in the muscle of the altitude resident (table IV), was quite unexpected in its magnitude. It is

presumed that, in combination with the lower barometric pressure, the ambient cold plays an important role in the rather complicated process of adaptation to high-altitude environments. It has been shown that the activity of the TPNH-cytochrome C reductase system is significantly increased in the microsomal fraction of the liver of cold-exposed hamsters (13).

No appreciable changes were observed in the TPN-isocitric dehydrogenase activity in relation to high-altitude exposure, either in the mitochondrial or in the supernatant fractions. Furthermore, there was no difference between the activity of the lactic dehydrogenase of high-altitude and sea-level residents, sug-

gesting that the glycolytic enzymes are not significantly involved in the adaptative process to high altitude. The increased activity of the DPNH-oxidase system, as well as that of the mitochondrial transhydrogenase, are suggestive of a higher rate of oxygen utilization through enzymatic pathways linked preferentially with the production of high-energy phosphate bonds (14); whereas, the higher rate of electrons flowing through the TPNH-cytochrome C system could presumably be related with the processes of cold adaptation (13). Higher concentrations of myoglobin in the muscle of man native to high altitude could favor the kinetics of oxygen utilization during the complicated phenomenon of adaptation to high altitudes.

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2. High-altitude adaptation
3. Enzyme chemistry
- I. AFSC Project 7758, Task 59582
- II. Contract No. AF 41(657)-249
- III. Institute of Andean Biology
- IV. Reynafarje, B.
- V. In ASTIA collection

hydrogenase, and isocitric and lactic dehydrogenases were also examined. A significantly higher myoglobin concentration was found in the muscle of the high-altitude natives as compared with sea-level residents. The enzyme systems DPNH-oxidase, TPNH-cytochrome C reductase, and transhydrogenase similarly showed a significantly higher activity in altitude residents. It was concluded that the respiratory capacity of the muscle was apparently higher in natives living at high altitude than in those living at sea level. The enhanced enzymatic activity was probably related to the higher pigment content of the skeletal muscle. Results on myoglobin determinations in several other muscles from certain sea-level patients are discussed.

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