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Blood Chemistry, Acid-Base, Electrolyte, Blood Lactate Metabolism and Sleepat 3480 m in Mountain Marathon Runners

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ABSTRACT

Altered blood chemistry, acid-base and electrolyte are suggested determinants of sleep disturbance, with frequent arousal at high altitude even in well and long-trained altitude marathon runners. In this sample of experienced altitude marathon runners with maximal aerobic power at sea level of 61.4 ± 2.7 ml/kg^{-1}·min^{-1} we found that pO_2 and percent of oxygen saturation (%SO_2) were lower at 2050 m and 3480 m than at sea level; pO_2 was higher after 38 - 41 hours than after 30 - 31 hours of acclimatization at 3480 m ($P < 0.05$). After ascentto 3480 m %SO_2 decreased ($P < 0.003$). Compared to sea level values, pH increased at high altitude ($P < 0.05$) consistent with changes in pCO_2 and $\left[HCO_3^-\right]$ ($P < 0.05$). Nocturnal %$SpaO_2$ at a sleeping altitude of 3480 m was lower ($P < 0.05$) than at sea level. At high altitude, the percent of wake (W) time and delay falling asleep (DFA) increased, whereas non-rapid eye movement sleep (N-REM), REM sleep and total sleep time (TST) decreased ($P < 0.05$). Simple regression analysis disclosed a significant correlation between the changes in TST and the percent of REM sleep and the changes in %$SpaO_2$ recorded during sleep ($P < 0.05$). Simple regression analysis showed a positive correlation between the changes in pO_2 at higher altitude and the percent of W and of TST ($P < 0.05$). The changes in pO_2, tCO_2 and $\left[HCO_3^-\right]$ correlated negatively and significantly with the percent of REM sleep changes at high altitude ($P < 0.05$). The TST changes at high altitude correlated positively with the changes in pO_2 and pH and correlated negatively with the changes in %SO_2, pCO_2, tCO_2, and $\left[HCO_3^-\right]$ ($P < 0.05$). The changes in the percent of W at high altitude correlated significantly and positively with the changes in bases excess [BE] at high altitude ($P < 0.05$). The changes in the percent of REM sleep correlated significantly and positively with the changes in [iCa^{++}] and [BE] and negatively with the changes in buffered bases [BB] and [BEeffective] ($P < 0.05$). The change in the percent of NREM + REM sleep at high altitude correlated significantly and positively with the changes in [BE] and [BB] concentration ($P < 0.05$). The increase in DFA at high altitude correlated significantly and negatively with the changes in pCO_2 and significantly and negatively with the changes in [K^+] ($P < 0.05$). Simple regression analysis demonstrated that the changes in pH at high altitude correlated positively and significantly with the percent of W and the DFA and negatively with the percent of changes in NREM sleep, REM sleep, NREM + REM sleep ($P < 0.05$). The decrease in the TST at high altitude correlated significantly and negatively with the changes in pCO_2, tCO_2, $\left[HCO_3^-\right]$ and [K^+] ($P < 0.05$). Our data demonstrate that the arterialized ear lobe techniques we used for evaluating most of the changes in blood chemistry, acid-base, electrolyte and blood lactate metabolism aresuitable for clinical and laboratory assessment and are important predictors of the quality and quantity of acclimatization and sleep at high altitude.

Keywords: Clinical Investigation at High Altitude; Laboratory Investigation at High Altitude; Hematochemistry; Acid-Base Metabolism; Electrolyte Metabolism; Blood Lactate Metabolism; Hydration Status; Sleep Quality Indicators; EEG; Mountain Marathon Runners

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1. Introduction

Clinical and laboratory investigations can help us to understand how the body adjusts its biochemical and physiological cellular needs to counter health risks associated with exposure and activities in hypobaric-hypoxia conditions. Research into these mechanisms is fundamental for studying the appropriate time course of acclimatization and the quality and quantity of sleep at high altitude [1-5].

Increasingly popular as a recreational sport, endurance and marathon race events at high altitude (2500 - 5500 m) became part of the Olympic games in 1968. Mountain running competitions are generally long-distance races similar to the classic marathon race, over rough terrain at high altitude, under relatively hypoxic conditions, with different gradients between the start and the end of the race.

Usually referred to as mountain marathon sky runners, some athletes possess the anthropometric characteristics typical of marathoners [6]. Experimental studies on the exercise physiology of mountain marathon runners demonstrated that marathon performance at high altitude is safe, demands strenuous effort, and induces transient, psychological, hematological and hormonal variations that fully resolve within 24 hours [6-16].

Studies of hypobaric-hypoxic high-altitude conditions have reported different degrees of high-altitude-induced altered states of consciousness [16,17]: insomnia, dizziness, sleep-wake disturbances [18], paroxysm [19] in normal subjects and mountain climbers and cognitive changes in mountain marathon runners [16].

Fundamental for attaining maximal performance in mountain marathon running at high altitude is the degree of acclimatization and training [7-16]. Current debate centers on the use of specific metabolic markers [3-5] and sleep quality and quantity as indices of general and central nervous system health status [2], as well as the tempo of acclimatization to high altitude conditions in mountain marathon runners preparing for performance at high altitude.

We studied healthy mountain marathoners native to sea level, matched for aerobic power, anthropometrical characteristics and level of athletic performance at high altitude under a controlled standard method/protocol for physical and psychological stressors. For clinical and laboratory investigation we took repeated measurement of metabolic variations in acid-base, electrolyte and blood lactate. The nocturnal percent of peripheral arterial oxygen saturation was measured every 30 seconds, and polysomnographic activity during sleep was recorded by means of electroencephalogram (EEG), electro-oculogram (EOG) and electromyogram (EMG) and by administration of questionnaires investigating sleep quality. The aim of the study was to collect data on the degree and *tempo of acclimatization* after ascent to 3480 m, which might be, together with nutrition, energetics [20], and exercise level [6], a crucial step in acclimatization when mountain marathon runners need to perfect their physical work capacity and performance during races at altitudes between 2000 m and 5000 m.

2. Materials and Methods

2.1. Subjects

Six healthy male mountain marathon runners (age range 37 - 44 years) were recruited for the study. The subjects were part of a group of experienced high-altitude runners that had been clinically tested since 1994. The mean body mass was 65.8 ± 4 kg; the height was 1.76 ± 0.37 cm; the mean maximal aerobic power at sea level was 61.4 ± 2.7 ml/kg^{-1}·min^{-1}. Maximal aerobic power was measured by using open circuit spirometry during a step-incremental treadmill run to volitional exhaustion.

Diet was controlled for food and water intake at low and at high altitudes during the study period; no neurodepresssive or neuroactive beverages were allowed. The subjects were also asked to avoid aerobic and anaerobic exercise about 12 hours before the sea level testing session and during the long testing sessions at high altitude. All experiments were carried out in accordance with the Helsinki Declaration (1975/1983) and the 86/609/EEC directive for experimental human care and designed to reduce stress and suffering to a minimal level, number of subjects used, and number of tests performed. Importantly, informed consent was obtained from each subject before each experiment, in accordance with the rules of the International Federation of Sport at Altitude (FSA).

Local time and barometric pressure (P_B) at an altitude of 122 m (Department of Clinical Science, Luigi Sacco Hospital, University of Milan, Milan, Italy), at 2050 m (Cervinia, Aosta, Valle d'Aosta, Italy) and at 3480 m (Plateau Rosà, Cervinia, Aosta, Valle d'Aosta, Italy) were measured using a chronometer and barometer sensors mounted on a mobile hemogasanalyzer kindly provided by OptiCCA®, Roche Diagnostics (Monza, Italy).

The mountain marathon runners ascended from 122 m at 11:00 h, reaching 2050 m at 13:00 - 14:00 h by car and 3480 m at about 15:00 - 16:00 h by cable car. Metabolic measurements were taken with the subjects in a behavioral wakeful state, preferably supine, otherwise in a sitting position, after at least 3 hours of rest, and before, during and after sleep in a wooden room.

2.2. Hematochemical, Acid-Base and Electrolyte Metabolism

In five mountain marathon runners, hematochemical pa-

rameters were measured in capillary blood drawn from the earlobesand in venous blood drawn from the brachial vein using heparinized capillaries and syringes. In the preanalytical phase, care was always taken to avoid as far as possible physical or chemical alteration of blood composition during sampling and while injecting the samples into the analyzer almost immediately after sampling. All analyses were performed on a portable hemogasanalyzer with throwaway cartridges (kindly provided by OptiCCA®, Roche Diagnostics, Monza, Italy). During the final data evaluation, not all the cartridge cells were found to be perfect for correct detection of chemicals due to mistakes in transporting the kit at the correct temperature for working at high altitude.

Sea level. Sampling was done with the subjects in a wakeful behavioral state at 122 m (11:19 ± 0.08 h; P_B 742 ± 7.7 mm Hg). *Capillary arterialized blood samples* from the earlobe were collected to measure partial oxygen pressure (pO_2; mm Hg), hemoglobin saturation (SO_2 [%]), partial carbon dioxide pressure (pCO_2; mm Hg), carbon dioxide content (tCO_2; mmol/L), hemoglobin content (tHb; g/dL), pH, bicarbonate concentration ($\left[HCO_3^-\right]$; mmol/L),the electrolytic pattern concentrations of sodium ($[Na^+]$; mmol/L), potassium ($[K^+]$; mmol/L), ionized calcium ($[iCa^{++}]$; mmol/L) and bases excess ([BE]; mmol/L), buffered bases ([BB]; mmol/L), bases excess active ([BE act]; mmol/L) and bases excess effective ([BEeffect]; mmol/L).

High altitude. Capillary blood samples were collected with the subjects awake on reaching an altitude of 2050 m (13:02 ± 1.09 h; P_B 587.6 ± 0.22 mm Hg), after 6 hours of acclimatization at 3480 m (21:42 ± 0.13 h; P_B 491 ± 7.9 mm Hg), following 30 - 32 hours of acclimatization at 3480 m (22:30 ± 0.9 h; P_B 495.7 ± 3.7 mm Hg), then at a sleeping altitude of 3480 m after a night's sleep and 38 - 41 hours of exposure (9:06 ± 1.06 h; P_B 495.4 ± 3.19 mm Hg). Capillary blood samples at the final altitude were taken from the earlobes to measure pO_2, SO_2 [%], pCO_2, tCO_2, tHb, pH, $\left[HCO_3^-\right]$, the electrolytic pattern concentrations of $[Na^+]$, $[K^+]$, $[iCa^{++}]$, the [BE], [BB], [BE act] and [BE effect].

Venous blood samples at high altitude were collected with the subjects in the supine position in a wakeful state after about 3 hours of rest, 30 - 32 hours of acclimatization, before going to sleep, after a night's sleep, and 40 - 41 hoursafter reaching an altitude of 3480 m. The samples were taken to measure pO_2, SO_2[%], pCO_2, tCO_2, tHB, pH, $\left[HCO_3^-\right]$, $[Na^+]$, $[K^+]$ and $[iCa^{++}]$.

2.4. Blood Lactate Metabolism

In five mountain marathon runners blood lactate ($[La^-]$; mmol/L) was measured in capillary blood drawn from the earlobes, with the subjects in the supine or sitting position, in a wakeful state, after 3 hours of rest at 122 m, then just after reaching 2050 m, after (after 3 hours of rest) 6, 30 - 31, and 38 - 41 hours of acclimatization at an altitude of 3480 m. The lactate concentrations were measured on a portable analyzer (YSI 1500 Sport, L-Lactate Analyzer®, Yellow Springs, OH, USA).

2.5. Polysomnographic Recordings

Polysomnographic signals were recorded at low altitude at the Department of Clinical Science, Luigi Sacco Hospital, University of Milan, Milan, Italy, and between 30 - 31/38 - 41 hours of acclimatization at an altitude of 3480 m (V High Mountain Alpinist High-House, Plateau Rosà, Cervinia, Aosta, Valle d'Aosta, Italy). Electroencephalographic (EEGs, μV/cm) activities were recorded by means of Ag/AgCl EEG scalp electrodes placed on the scalp of both hemispheres of the frontal, parietal and occipital cortices according to the international 10 - 20 system. The Ag/AgCl electrodes were covered with gauze, filled with conductive Grass EEG betonies paste and fixed with collodion 5%.The resistance between the electrodes was 5 - 32 kΩ for each pair of electrodes. Electro-oculograms ($EOG_{L\ and\ R}$, μV/cm) were performed using small throwaway electrodes placed to the right and left sides of each eye in the right up-orbital and the left down-orbital regions. Submental electromyograms (EMG-Sub, μV/cm) were recorded by means of two cupping Ag/AgCl electrodes fixed at a maximum distance of 1 cm. Amplified EEGs signals were acquired from F_3-F_4; F_3-P_3; P_3-A_2; O_1-O_2, EOG_L and R and EMG-derivations at a frequency of 512 Hz sent to a computer equipped with software applications for on-line digitalization, recording and display of signals (Extensa 355, Texas Instruments; DaqBook-100, IO-Tec, Milan, Italy) and for subsequent off-line signal analysis (LabView, National Instruments®, Milan, Italy). In the six mountain marathon runners the percent of nocturnal peripheral arterial oxygen saturation ($\%SpaO_2$) was recorded every 30 seconds by means of a 8500 Nonin finger pulse oxymeter (Nonin Med. Inc. MN, USA) during sleep at 122 m and between 30 - 31 and 38 - 41 hours of acclimatization at an altitude of 3480 m and at an approximate P_B of 495 mm Hg.

2.6. Subjective Perception of Sleep Quality

After polysomnographic recording at sea level and at high altitude, the subjects completed a questionnaire investigating perceived sleep quality according to the following indicators: 1) sleep latency (minutes of delay in falling asleep); 2) total number of hours slept; 3) awaking time before expected; 4) number of arousals; 5) whether or not rested on waking; 6) 10-point visual analogue scale (VAS) scored from poor (1) to excellent (10).

2.7. Statistical Analysis

Data are presented as the mean ± standard deviation (SD). Measurements were submitted to statistical analysis employing one way analysis of variance (ANOVA) followed by post-hoc multiple comparison of the means by applying Fisher's Protected Least Significant Difference (PLSD), Scheffé and Dunnet. Differences between paired and unpaired single or repeated measurements were also analyzed using Student's *t*-test. Linear simple regression analysis was also performed. Probability levels of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Hematochemical and Acid-Base Metabolism

The results of capillary and venous blood analysis showed changes in respiratory alkalosis and metabolic compensation during 6, 30 - 31, 40 - 41 hours of acclimatization at 3480 m. Changes in hemoglobin values were observed within the normal range for this type of athletes (**Tables 1-3**).

Table 1. Hematochemical, acid base, electrolyte, and blood lactate values at 122 m, during acclimatisation at 2050 m and after 6, 30 - 31, 38 - 41 hours of acclimatisation at an altitude of 3480 m. Data from capillary earlobe samples (1,2,3,4).

Parameters	122 m			2050 m				3480 m (6 h)				
							vs 122 m				vs 122 m	vs 2050 m
	No. (5)	Mean	SD	No. (5)	Mean	SD	P t-test	No. (5)	Mean	SD	P t-test	P t-test
P_B mmHg	4	742.1	7.7	5	587.6	0.2	0.0001	5	491.3	7.89	0.0001	0.0001
pO_2 mmHg	4	71.4	6	5	58.18	2	0.02	5	39.89	4.5	0.0002	0.00025
pCO_2 mmHg	4	41.21	3.38	5	34.74	3.3	0.03	5	33.65	0.85	0.01811	
tCO_2 mmol/L	4	29.87	1.44	5	27.22	1.8	0.04	5	27.16	1.37	0.02698	
SO_2%	4	93.9	2.19	5	91.321	1.5		5	77.7	6.3	0.0028	0.00712
tHb g/L	4	15.72	1.45	5	16.62	1.1		5	15.64	1.95		
pH	4	7.46	0.02	5	7.4954	0	0.03	5	7.5074	0.022	0.00791	
$[HCO_3^-]$ mmol/L	4	28.63	1.39	5	26.14	1.8	0.05	5	26.12	1.331	0.03183	
$[Na^+]$ mmol/L	1	139		5	138.96	1.6		2	141.5	0.771		
$[K^+]$ mmol/L	1	4.7		5	5.06	0.9		2	4.25	0.007		
$[iCa^{++}]$ mmol/L	1	1.17		5	1.19	0		2	1.19	0.014		
[BE] mmol/L	4	4.35	0.77	5	3.3	1.3		5	3.54	1.6		
[BB] mmol/L	n.r.	n.r.	n.r.	5	52.02	1.6		n.r.	n.r.	n.r.		
[BEact] mmol/L	n.r.	n.r.	n.r.	5	3.82	1.3		n.r.	n.r.	n.r.		
[BEeffect] mmol/L	n.r.	n.r.	n.r.	5	3.7	2.7		n.r.	n.r.	n.r.		
$[La^-]$ mmol/L	3	0.77	0.1	5	0.96	0.2		5	0.88	0.2419		

Parameters	3480 m (30 - 31 h)						3480 m (38 - 41 h)						
				vs 122 m	vs 2050 m	vs 3480 m (6 h)				vs 122 m	vs 2050 m	vs 3480 m (6 h)	vs 3480 m (30 - 31 h)
	No. (5)	Mean	SD	P t-test	P t-test	P t-test	No. (5)	Mean	SD	P t-test	P t-test	P t-test	P t-test
P_B mmHg	10	495.7	3.4	0.0001	0.0001		10	495.4	3.18	0.0001	0.0001		
pO_2 mmHg	10	45.32	4.22	0.00105	0.0001	0.0565	10	49.49	4.03	0.0022	0.0001	0.0045	0.0366
pCO_2 mmHg	10	29.33	2.61	0.0021	0.0167	0.0004	10	28.03	2.33	0.0016	0.0066	0.0001	
tCO_2 mmol/L	10	83.55	4.82	0.00015	0.0005		10	86.45	3.51	0.0009	0.0025	0.0322	
SO_2%	10	24.25	1.81	0.00049	0.0172	0.0057	10	22.77	1.75	0.0001	0.002		
tHb g/L	10	15.90	1.5				10	16.41	1.29			0.0003	
pH	10	7.52	0.02	0.00075			10	7.511	0.03	0.0001			
$[HCO_3^-]$ mmol/L	10	23.36	1.73	0.00058	0.0196	0.0063	10	21.92	1.7	0.0001	0.0002	0.00036	
$[Na^+]$ mmol/L	7	141.3	1.49	0.0337			7	139.6	1.36				0.0445
$[K^+]$ mmol/L	7	5.23	0.63		0.0057		7	5.23	0.67		0.0079		
$[iCa^{++}]$ mmol/L	7	1.21	0.04				7	1.204	0.06				
[BE] mmol/L	10	1.68	1.42	0.00104	0.058		10	0.41	1.57		0.0041	0.00412	
[BB] mmol/L	5	50.54	1.49				4	49.33	2.32				
[BEact] mmol/L	5	2.18	1.4				4	1.625	1.13		0.0299		
[BEeffect] mmol/L	5	1.34	1.15				4	1.925	1.44				
$[La^-]$ mmol/L	5	0.92	0.36				5	0.601	0.25		0.0498		

(1) Abbreviations of haematochemical parameter and units are detailed in the method sections. (2) Measurements were submitted to statistical analyses, employing one-way analyses of variance ANOVA followed by post-hoc multiple comparisons of the meansby applying Fisher's and Scheffe's Protected Least Significance Difference (PLSD) and Student t-test. The significant level was set at $P < 0.05$. (3) Measurements submitted to statistical Dunnet analysis were found not significantly different. (4) Only the detailed values regarding the Student t-test are reported. (5) No = Number of measurements performed in the five mountain runners at different altitudes.

Table 2. Delta of hematochemical, acid base, electrolyte, and blood lactate values at 122 m, during acclimatisation at 2050 m, and after 6, 30 - 31, and 38 - 41 hours at an altitude of 3480 m. Data from capillary earlobe samples.

Parameters	122 m			
	vs 2050 m	vs 3480 m (6 h)	vs 3480 m (30 - 31 h)	vs 3480 m (38 - 41 h)
P_B mmHg	−154.5	−250.8	−264.4	−246.7
pO_2 mmHg	−13.22	−31.51	−26.08	−21.91
pCO_2 mmHg	−6.47	−7.56	−11.88	−13.18
tCO_2 mmol/L	−2.65	−2.71	−5.62	−7.1
SO_2%	−2.58	−23.2	−8.35	−7.45
tHb g/L	0.9	−0.08	0.18	0.69
pH	0.0359	0.0479	0.069	0.0516
$\left[HCO_3^-\right]$ mmol/L	−2.485	−2.505	−5.265	−7.335
[BE] mmol/L	−1.05	−0.81	−2.67	−3.94
[La^-] mmol/L	0.26	0.18	0.22	0.099

Parameters	2050 m		
	vs 3480 m (6 h)	vs 3480 m (30 - 31 h)	vs 3480 m (38 - 41 h)
P_B mmHg	−96.3	−91.9	−92.2
pO_2 mmHg	−18.29	−12.86	8.69
pCO_2 mmHg	−1.09	−5.41	−6.715
tCO_2 mmol/L	−0.06	−2.97	−4.45
SO_2%	−20.62	−5.77	−4.78
tHb g/L	−0.98	−0.72	−0.21
pH	0.012	0.025	0.0157
$\left[HCO_3^-\right]$ mmol/L	−0.02	−2.78	−4.85
[Na^+] mmol/L	2.54	2.33	0.61
[K^+] mmol/L	−0.81	0.17	0.17
[iCa^{++}] mmol/L	0	0.02	0.0142
[BE] mmol/L	0.24	−1.62	−2.89
[BB] mmol/L	−2.38	−3.6875	
[BEact] mmol/L	−1.64	−2.195	
[BEeffect] mmol/L	−2.36	−1.775	
[La^-] mmol/L	−0.08	−0.04	−0.359

Parameters	vs 3480 m (6 h)	
	vs 3480 m (30 - 31 h)	vs 3480 m (38 - 41 h)
P_B mmHg	4.4	4.1
pO_2 mmHg	5.43	9.6
pCO_2 mmHg	−4.32	−5.62
tCO_2 mmol/L	−2.91	−4.39
SO_2%	14.85	15.75
tHb g/L	0.26	0.77
pH	0.013	0.0037
$\left[HCO_3^-\right]$ mmol/L	−2.76	−4.83
[BE] mmol/L	−1.85	−3.13
[La^-] mmol/L	0.04	−0.279

Parameters	3480 m (30 - 31 h)
	vs 3480 m (38 - 41 h)
P_B mmHg	−0.3
pO_2 mmHg	4.17
pCO_2 mmHg	−1.305
tCO_2 mmol/L	−1.48
SO_2%	0.9
tHb g/L	0.51
pH	−0.0093
$\left[HCO_3^-\right]$ mmol/L	−2.07
[Na^+] mmol/L	−1.72
[K^+] mmol/L	0
[iCa^{++}] mmol/L	−0.00058
[BE] mmol/L	−1.27
[BB] mmol/L	−1.3075
[BEact] mmol/L	−0.555
[BEeffect] mmol/L	0.585
[La−] mmol/L	−0.319

Table 3. (a) Differences in hematochemical, acid-base and electrolyte values observed after 30 - 31 and 38 - 41 hours of acclimatisation at an altitude of 3480 m. All data from venous blood samples. (b) Delta between the hematochemical, acid base and electrolytic capillary earlobe values (Table 1) observed after 30 - 31 and 38 - 41 hours of acclimatisation at an altitude of 3480 m versus those observed in venous blood samples (Table 3(a)); (c) Results of one-way ANOVA of capillary earlobe values (Table 1) and venous blood values (Table 3(a)) observed after 30 - 31 hours of acclimatisation at an altitude of 3480 m; (d) Results of one-way ANOVA of capillary earlobe values (Table 1) and venous blood values (Table 3(a)) observed after 38 - 41 hours of acclimatisation at an altitude of 3480 m.

(a)

Venous Blood Samples								
Parameters	**3480 m (30 - 31 h)**			**3480 m (38 - 41 h)**				
	No subjects	**Mean**	**SD**	**No subjects**	**Mean**	**SD**	**Delta**	**P t-test**
P_B mmHg	6	496	6.32	5	498	5.77	2.4	0.53
pO_2 mmHg	5	40.4	9.78	5	44.12	12.87	3.75	0.42
pCO_2 mmHg	6	39.9	5.21	5	37.32	4.74	−2.58	0.42
tCO_2 mmol/L	6	77.5	13.69	5	78.86	16.25	1.36	0.89
SO_2%	6	26.58	2.56	5	25.87	1.99	−0.71	0.58
tHb g/L	6	14.2	2.49	5	15.78	1.53	1.58	0.26
pH	6	7.4167	0.02	5	7.434	0.05	0.0173	0.42
$\left[HCO_3^-\right]$ mmol/L	6	25.33	2.39	5	24.68	1.89	−0.65	0.6
$[Na^+]$ mmol/L	6	144.2	4.9	5	141.5	2.31	−2.7	0.29
$[K^+]$ mmol/L	6	4.49	0.32	5	4.46	0.1	−0.03	0.85
$[iCa^{++}]$ mmol/L	6	1.27	0.1	5	1.22	0.05	−0.05	0.4

(b)

Parameters	**Delta 30 - 31 h**	**Delta 38 - 41 h**
pO_2 mmHg	−4.92	−5.37
pCO_2 mmHg	10.57	9.295
tCO_2 mmol/L	2.33	3.1
SO_2%	−8.05	−7.59
tHb g/L	−1.7	−0.63
pH	−0.1037	−0.0771
$\left[HCO_3^-\right]$ mmol/L	1.97	3.39
$[Na^+]$ mmol/L	2.91	1.93
$[K^+]$ mmol/L	−0.74	−0.77
$[iCa^{++}]$ mmol/L	0.06	0.0158

(c)

Parameters	**DF**	**F**	**P**	**Fisher PLSD**	**Sheffe'**	**Dunnet**
pCO_2 mmHg	1,15	29.8	0.0001	4.158	29.8	5.456
SO_2%	1,12	25.2	0.003	6.208	25.2	5.019
tCO_2 mmol/L	1,15	4.6	0.05	2.334	4.6	2.145
pH	1,15	95.5	0.0001	0.023	95.53	9.771

(d)

Parameters	**DF**	**F**	**P**	**Fisher PLSD**	**Sheffe'**	**Dunnet**
pCO_2 mmHg	1,14	27.003	0.0002	3.856	27.003	5.196
tCO_2 mmol/L	1,12	10.741	0.0074	10.825	10.741	3.277
SO_2%	1,14	9.044	0.0101	2.162	9.044	3.007
pH	1,14	18.002	0.001	0.039	18.002	4.243
$\left[HCO_3^-\right]$ mmol/L	1,14	7.838	0.015	2.084	7.838	2.8

There was a statistically significant decrease between pO_2 (mm Hg) values measured at 3480 m and at a P_B of approximately 495 mm Hg and those observed at 122 m and at P_B of approximately 742 mm Hg (**Tables 1-3**). pO_2 values measured after 30 - 31 hours and after 38 - 41 hours of acclimatization and at a P_B of approximately 495 mm Hg were both significantly higher than those after 6 hours at an altitude of 3480 m and at a P_B of approximately 491 mm Hg; pO_2 values increased between 30 - 31 hours and 38 - 41 hours of acclimatization at 3480 m and at a P_B of approximately 495 mm Hg (**Tables 1-3**).

Both pCO_2 (mm Hg) and $\left[HCO_3^-\right]$ (mmol/L) significantly decreased at higher altitudes (**Tables 1-3**); pCO_2 and $\left[HCO_3^-\right]$ values were significantly higher at 2050 m than those at 30/31 - 40/41 hours after reaching 3480 m. A decrease was also noted between measurements taken at 30 - 31 and 38 - 41 hours at 3480 m versus those after 6 h at 3480 m (**Tables 1-3**). The $\left[HCO_3^-\right]$ concentration after 38 - 41 hours of acclimatization at 3480 m was lower than that measured after 30 - 31 hours at 3480 m (**Tables 1-3**).

There was a statistically relevant increase in pH at high altitude compared to that measured at 122 m (**Tables 1-3**). Comparison of the pH values at 2050 m with those recorded at 3480 m still showed an increase between measurements taken after 6 and 30 hours of high-altitude exposure. Although other changes were not statistically significant, pH values tended to decrease after 38 - 41 hours of high-altitude exposure (**Tables 1** and **2**).

There was a significant linear decrease in all types of bases [BE], [BB], [BE active], [BE effective] (mmol/L) at high altitude (**Tables 1** and **2**).

No significant changes were noted in the average hematochemical values of venous blood samples collected from the brachial vein between 30 - 31 and 38 - 41 hours of acclimatization at 3480 m (**Table 3**).

Nevertheless, there were statistical differences between SO_2[%], pCO_2, tCO_2 (mmol/L) and pH levels in capillary and venous blood samples collected after 30 - 31 hours of acclimatization at 3480 m (**Table 3**). There were also statistically significant differences between the SO_2[%], pCO_2, tCO_2, pH and $\left[HCO_3^-\right]$ in capillary and venous blood samples taken after 38 - 41 hours of acclimatization at 3480 m (**Table 3**).

3.2. Electrolyte Metabolism

Changes in electrolyte balance were determined by measuring the concentration of sodium $[Na^+]$ (mmol/L), potassium $[K^+]$ (mmol/L) and ionized calcium $[iCa^{++}]$ (mmol/L). The normal average electrolyte values barely changed at high altitude (**Tables 1-3**); the $[Na^+]$ and $[K^+]$ varied significantly at high altitude (**Tables 1** and **2**).

3.3. Blood Lactate Metabolism

During the waking state at low altitude, after reaching 2050 m, and after 6, 30 - 31 and 38 - 41 hours of acclimatization at an altitude of 3480 m, the capillaryblood lactate values were consistently less than 2 mmol/L. Nonetheless, after 38 - 41 hours of acclimatization and two nights of sleep at 3480 m, the lactate values were significantly lower than those measured at 2050 m (**Tables 1** and **2**).

3.4. Polysomnographic Recordings

3.4.1. EEG Recordings

Sleep at 3480 m was characterized by increased wakefulness ([W] 38.8%; $P_{t\text{-test}} < 0.01$) and a parallel significant decrease of 28% in non-REM sleep (NREM) ($P_{t\text{-test}} < 0.0311$) and of 11% in rapid eye movement (REM) sleep ($P_{t\text{-test}} < 0{,}008$). The total sleep time ([TST]; $P_{t\text{-test}} < 0.01$) decreased by 38.5% (**Table 4**).

3.4.2. Percentage of Peripheral Arterial Oxygen Saturation during Sleep

The average $\%SpaO_2$ was significantly lower at a sleeping altitude of 3480 m than at sea level ($P_{t\text{-test}} < 0.001$) (**Table 4**).

3.4.3. Subjective Perception of Sleep Quality

We found that 65% of subjects took longer than their normal time to fall asleep (delay in falling asleep [DFA]; **Table 4**); 30% woke up 2 hours before the expected time and 60% woke up more than once. Sleep quality was perceived as significantly poorer at 3480 m than at 122 m (5.58 ± 1.8 vs 7.84 ± 1.47; $P < 0.05$); sleep duration was shorter and 60% of subjects felt poorly restored on waking up.

3.4.4. ANOVA of Quantity and Quality Sleep and $\%SpaO_2$ Changes

ANOVA revealed significant differences in $\%SpaO_2$ values, the percent of W, NREM, REM, NREM + REM sleep, and in TST between measurements taken at 122 m and those at an altitude at 3480 m (**Table 4(b)**).

3.5. Other Statistical Analyses

3.5.1. Simple Regression Analysis of Polysomnographic Data and the $\%SpaO_2$ Recorded during Sleep

Simple regression analysis of the percent of W, NREM, REM, NREM + REM sleep and of TST disclosed a significant correlation between the changes in TST and the percent of REM sleep and the $\%SpaO_2$ changes recorded during sleep (**Table 4(c)**).

Table 4. (a) Changes in the percent of nocturnal peripheral arterial oxygen saturation (%SpaO2), Wake (W), Non-Rapid Eye Movement (%NREM) sleep, Rapid Eye Movement (%REM), Total Sleep time (TST, h) and Delay in Falling Asleep (DFA, s) observed during sleep at 122 m and between 30 - 31 and 38 - 41 hours of acclimatization at an altitude of 3480 m. (b) Nocturnal %SpaO$_2$, %W, %NREM, %REM, %NREM + REM, TST (h) and DFA (s) recorded at 122 m and at 3480 m resulted significantly different at one way ANOVA for each measurement. (c) Simple regression analysis between the nocturnal %SpaO$_2$, %REM sleep, and TST (h) resulted significant.

(a)

	122 m		3480 m	
Parameters	**Mean**	**SD**	**Mean**	**SD**
%SpaO$_2$	95.6	0.85	80	3.64
%W	19.5	10.4	58.3	29.9
%NREM	63	8	34.7	26.5
%REM	17.5	5	7.2	5.8
%NREM + REM	80.5	10.6	41.8	30
TST (h)	7:31:50	0:43:57	4:53:15	1:53:15
DFA (s)	349.5	383.5	693.7	616.1

(b)

Parameters	**DF**	**F**	**P**	**Fisher PLSD**	**Sheffe'**	**Dunnet**
%SpaO$_2$	1,10	105.5	0.0001	3.4	105.5	10.25
%W	1,11	9.03	0.0132	28.8	9.03	3
%NREM	1,12	6.28	0.0311	25.2	6.28	2.51
%REM	1,13	10.88	0.008	6.98	10.88	3.3
%NREM + REM	1,14	9.03	0.0132	28.68	9.03	3
TST (h)	1,15	12.11	0.05	1.7	12.11	3.48
DFA (s)	1,16	1.35	0.2723	660.17	1.35	1.16

(c)

Parameters	**Observations**	**DF**	**R-sqared**	**Coefficient**	**F**	**P**	**t**
%SpaO$_2$							
%REM	12	1,11	0.45	−39.07	8.09	0.0174	2.84
TST (h)	12	1,11	0.74	−10.74	28.393	0.0003	5.329

3.5.2. Simple Regression Analysis of Changes in Metabolic Components of Capillary Blood Analysis and Sleep

Simple regression analysis showed a positive correlation between the changes in pO_2 at higher altitude and the percent of W and of TST (**Table 5**). The changes in pO_2, tCO_2 and $\left[HCO_3^-\right]$ correlated negatively and significantly with the percent of REM sleep changes at high altitude (**Table 5**). The TST changes at high altitude correlated positively with the changes in pO_2 and pH and correlated negatively with the changes in SO_2[%], pCO_2, tCO_2, and $\left[HCO_3^-\right]$ (**Table 5**).

The changes in the percent of W at high altitude correlated significantly and positively with the [BE] changes at high altitude (**Table 6**). The changes in the percent of REM sleep correlated significantly and positively with the changes in [iCa^{++}] and [BE] and negatively with the changes in [BB] and [BEeffective] (**Table 6**). The change in the percent of NREM+REM sleep at high altitude correlated significantly and positively with the changes in [BE] and [BB] concentration (**Table 6**).

3.5.3. Simple Regression Analysis of Changes in Venous Blood Metabolic Components and Sleep

The increase in DFA at high altitude correlated significantly and negatively with the changes in pCO_2 and significantly and negatively with the changes in [K^+] in venous blood (**Table 7**). Simple regression analysis demon-

Table 5. Results of simple regression analysis between pO_2, $\%SO_2$, pCO_2, tCO_2, pH and $[HCO_3^-]$ values in earlobe capillary blood samples taken after 30 - 31 and 38 - 41 hours of acclimatization and %Wake (W), Rapid Eye Movement (REM) sleep and Total Sleep Time (TST, h) recorded between 30 - 31 and 38 - 41 hours of acclimatization at 3480 m.

Parameters	Observations	DF	R−squared	Coefficent	F	P	t
pO_2 mmHg							
%W	10	1.9	0.441	125.963	6.32	0.0362	2.513
TST	10	1.9	0.479	0.719	7.35	0.0266	2.711
$\%SO_2$							
TST	10	1.9	0.449	−13.745	7.959	0.0225	2.821
pCO_2 mmHg							
TST	10	1,9	0.56	−0.587	10.19	0.0128	3.192
tCO_2 mmol/L							
%REM	10	1.9	0.47	−29.938	7.083	0.0287	2.661
TST	10	1.9	0.619	−4.912	12.976	0.007	3.602
pH							
TST	10	1.9	0.56	23.639	6.704	0.0322	2.589
$[HCO_3^-]$ mmol/L							
%REM	10	1.9	0.476	−31.226	7.255	0.0273	2.694
TST	10	1.9	0.615	−5.139	12.786	0.0072	3.576

Table 6. Results of simple regression analysis between [iCa++], [BE], [BB] and [BEeffective] values from earlobe capillary blood samples taked during 30 - 31 and 38 - 41 hours of acclimatization and the percent of Wake (%W), Rapid Eye Movement (%REM) sleep, and NREM + REM (%NREM + REM) as measured by EEG between 30 - 31 and 38 - 41hours of acclimatisation at 3480 m.

Parameters	Observations	DF	R-squared	Coefficient	F	P	t
$[iCa^{++}]$ mmol/L							
%REM	7	1,6	0.708	182.193	12.145	0.0176	3.485
[BE] mmol/L							
%W	10	1,9	0.427	81.517	5.954	0.0406	2.44
%REM	10	1,9	0.557	0.01	10.056	0.0132	3.171
%NREM + REM	10	1,9	0.428	18.633	5.977	0.0403	2.445
[BB] mmol/L							
%REM	4	1,3	0.985	−228.309	128.272	0.0077	11.326
%NREM + REM	4	1,3	0.916	694.503	21.857	0.0428	4.675
[BEeffect] mmol/L							
%REM	4	1,3	0.91	−0.624	20.13	0.0463	4.487

Table 7. Results of simple regression analysis between pCO_2, tCO_2, pH, $[HCO_3^-]$ and [K+] values in venous blood samples collected. after 30 - 31 and after 38 - 41 hours of acclimatization at 3480 m and the percent of Wake (%W), Non-Rapid Eye Movement (%NREM sleep), Rapid Eye Movement sleep (%REM), Total Sleep Time (TST, h), Delay in Falling Asleep (DFA, s) recorded between 30 - 31 and 38 - 41 hours of acclimatisation at 3480 m.

Parameters	Observations	DF	R-squared	Coefficient	F	P	t
pCO_2 mmHg							
DFA	10	1,9	0.525	−3.28	8.842	0.0178	2.973
TST	10	1,9	0.415	−4.883	5.665	0.0445	2.38
tCO_2 mmol/L							
TST	10	1,9	0.751	−10.402	24.101	0.0012	4.909
pH							
%W	10	1,9	0.521	6.084	8.695	0.0185	2.949
%REM	10	1,9	0.429	−4.406	6.009	0.0399	2.451
%NREM	10	1,9	0.534	−1.528	9.179	0.0163	3.03
%NREM + REM	10	1,9	0.517	−5.934	8.569	0.0191	2.927
DFA	10	1,9	0.563	11.224	10.29	0.0125	3.208
$[HCO_3^-]$ mmol/L							
TST	10	1,9	0.758	−10.331	25.061	0.001	5.006
$[K^+]$ mmol/L							
DFA	7	1,6	0.689	−6.285	11.071	0.0208	3.327
TST	7	1,6	0.819	−19.784	22.579	0.0051	4.752

strated that the changes in pH at high altitude correlated positively and significantly with the percent of W and the DFA and negatively with the changes in the percent of NREM sleep, REM sleep, NREM + REM sleep (**Table 7**). The decrease in the TST at high altitude correlated significantly and negatively with the changes in pCO_2, tCO_2, $\left[HCO_3^-\right]$ and $[K^+]$ in venous blood.

4. Discussion

4.1. Technical Considerations

The metabolic aerobic power consumption values recorded in our subjects were averaged and found to be similar to those reported by Muller *et al*. [17]. Questions remain about the validity of blood gas values measured in arterialized earlobe blood instead of peripheral arterial blood samples for clinical and laboratory investigation. Recently, arterialized earlobe blood sampling has been demonstrated a simple and safe method suitable for clinical, laboratory molecular evaluation. The earlobe blood sampling technique has been reported to be less painful than the more correct arterial sampling and is advocated as a reliable method for arterial blood gas determination and as standard practice for acid-basic balance analysis [21]. The validity of arterialized earlobe blood samples has been shown for the determination of pCO_2 levels. Arterialized earlobe blood is said to be more suitable for clinical assessment of arterial pO_2 only for values <60 mm Hg and less suitable for higher values due to underestimation of the real value [21]. Overall, our study seems to confirm that the techniques we used for evaluating most of the changes in blood chemistry, acid-base equilibrium [21], electrolyte and blood lactate are suitable for clinical assessment and for metabolic research at high altitude.

4.2. Hematochemical and Acid-Base Equilibrium

In the mountain marathon runners in our study, the hematochemical alterations at high altitude resembled and followed the classic physiological time course of responses to the hypobaric-hypoxemia and hypocapnic state [3-5,20]. The physiological responses became noticeable after 30 - 31 hours to 40 - 41 hours at 3480 m. At low altitude the average pH was approximately 7.4595 ± 0.0139, slightly higher than that reported by Samaja *et al*. [5]. The average pH in the mountain marathon runners at an altitude of 3460 m after 6 - 41 hours of acclimatization was slightly higher than that reported in Caucasian control subjects exposed to approximately the same alti-

tude [3-5].

In the mountain marathon runners, the average pCO_2 at sea level was similar to that reported in by Samaja *et al*. [4]; after 41 hours of acclimatization and sleep at 3480 m, it was similar to that recorded in the Sherpas [4]. This study demonstrates that: 1) at 122 m the average pO_2 was similar to that previously reported in **Table 1** by Samaja *et al*. [3-5]; 2) the average pO_2 level observed in the mountain marathon runners after 6 hours at 3480 m was similar to that observed in the Caucasian subjects at 6450 m, and lower than that observed at 5050 m; 3) the average pO_2 in the mountain marathon runners after 41 hours of acclimatization was nearly similar to that observed in the Caucasians and Sherpas at 3400 m after acclimatization [4].

The average $\%SpaO_2$ in the mountain marathoners after 6 hours of acclimatization at 3480 m was similar to that observed at 6450 m by Samaja *et al*. and reached 83% after 30 - 31 hours of acclimatization at 3480 m, a value similar to what Samaja *et al*. [3,4] reported after 5 weeks of acclimatization at 5050 m. It rose to 86% after 41 hours of acclimatization at 3480 m, which was very near the average values (88%) that Samaja *et al*. recorded in Caucasian subjects at 5050 m after several days of acclimatization [3,4].

In agreement with Samaja *et al*. [3-5], we suggest that (as in their Caucasian subjects) the metabolic response to respiratory alkalosis may have slightly but significantly improved blood O_2 affinity and subsequently the blood pO_2 level in our subjects (**Table 1**).

The continuous decrease in pH, pCO_2 and bicarbonate values collected after a night's sleep and 41 hours of stay at 3480 m showed that compensation of respiratory alkalosis had been activated. Similar data were reported in a study conducted many years ago [22].

In line with data reported by Weil [18], the $\%SpaO_2$ measured in our subjects during sleep, and between 30 - 31 and 38 - 41 hours of acclimatization at 3480 m, fluctuated between 65% and 84% [23]. The average excess of bases [BE] recorded after 40 - 41 hours of acclimatization to high altitude was 0.41 mmol/L and was higher than that considered necessary for reaching a physiological pH value. Of the bases excess, the average $\left[HCO_3^-\right]$ closely followed the same trend as [BE], decreasing fairly consistently during acclimatization. In brief, in this study, a correct activation of compensation of respiratory alkalosis and acclimatization at 3480 m was demonstrated by the linear decrease in the bases excess. The decrease in [BE] was not sufficient to normalize the pH, even after 41 hours of acclimatization at 3480 m; nonetheless, it was probably the best value that could be reached at that altitude at a P_B of 490 mm Hg [3-5] and with the chosen exercise and dietary regimen [20].

4.3. Electrolyte Metabolism

While alterations were noted in acid-base metabolism, the concentration of $[Na^+]$, $[K^+]$ and $[iCa^{++}]$ ions remained within the normal range. We found that, under controlled conditions, the $[Na^+]$ increased after 6 hours of exposure to high altitude but returned to the values observed at 122 m within 41 hours of exposure at 3480 m.

4.4. Blood Lactate Metabolism

On average, blood lactate levels after a night's sleep and 41 hours of acclimatization at 3480 m were lower than those observed during the wakeful state. Our data show that the peripheral metabolic pathway was aerobic throughout acclimatization from 2050 m to 3480 m. The decrease in blood lactate values observed after 41 hours of acclimatization and a sleeping altitude of 3480 m suggests that the aerobic power of mountain marathon runners at rest improves [20,24].

4.5. Subjective Perception of Sleep Quality and Quantity

The decrease in the nocturnal $\%SpaO_2$ values at high altitude versus those measured at sea level significantly correlated with the significant decrease in total sleep time (TST) and %REM sleep, indicating that the measurement of nocturnal $\%SpaO_2$ taken every 30 seconds can be used as an index of sleep quantity and quality. It is also very important for evaluating the degree of acclimatization to high altitude and for indicating the risk of altered states of consciousness, as described by Finnegan *et al.* [19]. Importantly, unlike these authors, we found no paroxysmal figures on the EEG tracings.

In this study, one of the factors underlying the decrease in NREM and REM sleep was significantly correlated with the decrease in nocturnal $\%SpaO_2$. Altitude-induced low arterial oxygen pressure might have elicited cortical arousal via afferent chemoreceptor activation of the ascending reticular activating system located in the brain stem reticular formation. This mechanism may explain the decrease in NREM and REM sleep in the mountain marathon runners. In other words, at a barometric pressure of 495 mm Hg in the hypoxic-hypobaric conditions of this study, the nocturnal pO_2/pCO_2 ratio most likely may have played, cyclically, a critical role in activating directly and/or indirectly the bulbo-pontine and hypothalamic reticular activating system involved in the behavioral and metabolic integration of autonomic, cardiovascular and respiratory functions and in cortical arousal.

5. Conclusion

Overall, our results suggest that monitoring the time

course of changes in blood chemistry, acid-base balance, electrolyte and blood lactate metabolism during the wakeful state and the nocturnal %SpaO_2 during sleep may help to set new criteria for assessing how well mountain marathon runners acclimatize to high-altitude exposure and for predicting the quality of their refreshed sleep. Studying the time course of acclimatization to high altitude is a fundamental part of the correct physiological approach to exercise training at high altitude and essential for preparing athletes to live and exercise at high altitude.

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