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Effect of High Altitude on Protein Metabolism in Bolivian Children

JOSE L. SAN MIGUEL,[1] HILDE SPIELVOGEL,[1] JACQUES BERGER,[2] MAURICIO ARAOZ,[1] CARMEN LUJAN,[1] WILMA TELLEZ,[1] ESPERANZA CACERES,[1] PIERRE GACHON,[3] JEAN COUDERT,[4] and BERNARD BEAUFRERE[3]

ABSTRACT

San Miguel, Jose L., Hilde Spielvogel, Jacques Berger, Mauricio Araoz, Carmen Lujan, Wilma Tellez, Esperanza Caceres, Pierre Gachon, Jean Coudert, and Bernard Beaufrere. Effect of high altitude on protein metabolism in Bolivian children. *High Alt Med Biol* 3:377–386, 2002.—In Bolivia, malnutrition in children is a major health problem that may be caused by inadequate protein, energy, and micronutrient intake; exposure to bacterial and parasitic infections; and life in a multistress environment (high altitude, cold, cosmic radiation, low ambient humidity). However, no data on protein absorption and utilization at high altitude were available. Therefore, we evaluated the effect of altitude on protein metabolism in Bolivian children. We measured protein utilization using leucine labeled with a stable isotope (^{13}C) in two groups of healthy prepubertal children matched for age. Group 1 ($n = 10$) was examined at high altitude (HA) in La Paz (3600 m), and group 2 ($n = 10$) at low altitude (LA) in Santa Cruz (420 m). The nutritional status did not differ between groups but, as was to be expected, the HA group had higher hemoglobin concentration than the LA group. The children consumed casein that was intrinsically labeled with L-(1-^{13}C) leucine and expired $^{13}CO_2$ was analyzed. Samples of expired air were measured by isotope ratio mass spectrometer in Clermont-Ferrand. It was found that cumulative leucine oxidation ($^{13}CO_2$) at 300 min after ingestion was 19.7 ± 4.9% at HA and 25.2 ± 3.2% at LA. These results showed that protein absorption and/or utilization is significantly affected by altitude.

Key Words: protein turnover; resting metabolic rate; stable isotope; leucine oxidation

INTRODUCTION

ACUTE EXPOSURE TO high altitude (HA) (i.e., 3000 m above sea level) affects the nutritional status of human beings (Kayser, 1992). Weight loss is observed (Boyer and Blume, 1984), and this is initially caused by a loss of body water (Consolazio et al., 1968; Jain et al., 1980; Krzywicki et al., 1971; Hoyt et al., 1992; Milledge, 1992). Later, weight loss is due to a

[1]Instituto Boliviano de Biología de Altura, Facultad de Medicina, Universidad Mayor de San Andrés, P.O. Box 641 La Paz, Bolivia.
[2]Institut de Recherche pour le Developpement, IRD, Laboratoire de Nutrition Tropicale, 34032 Montpellier Cedex 1, France.
[3]Laboratoire de Nutrition Humaine, Centre Hospitalier Universitaire et Laboratoire de Physiologie et Biologie du Sport, Faculté de Médecine, Place Henri Dunant, 63001, Clermont-Ferrand cedex, France.
[4]Physiologie et Médecine du Sport, Faculté de Médecine, Clermont-Ferrand, France.
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reduction of fat tissue and muscle mass of up to 17% in the thigh (Boyer and Blume, 1984; Hoppeler et al., 1990; MacDougall et al., 1991).

During acclimation to HA, mountain climbers have access to limited quantities of appetizing food. Additionally, they commonly experience discomfort and suffer from anorexia (Boyer and Blume, 1984). Moreover, digestibility of foods and absorption of nutrients are decreased (Milledge et al., 1972; Rai et al., 1975; Boyer and Blume, 1984; Butterfield, 1990). Climbers have low energy intake (i.e., loss of appetite, poor digestibility) (Kayser, 1992); however, their basal metabolic rate is elevated and thus energy expenditure increases (Grover, 1963; Gill and Pugh, 1964; Butterfield, 1990). This generates an energy deficit that results in weight loss (Hannon and Sudman, 1973; Stock et al., 1978; Hannon, 1980; Rose et al., 1988; Butterfield et al., 1992).

Protein metabolism is also affected by acute exposure to hypobaric hypoxia. Leucine turnover and synthesis of glutamine and intermediates of the tricarboxylic acid cycle are decreased as a result of acute exposure to hypobaric hypoxia (Rennie et al., 1983; Wagenmakers, 1992). Supplementation with branched chain amino acids for individuals who have migrated to HA has been shown to prevent muscle loss (Schena et al., 1992). Experiments in animals have shown that acute hypoxia reduces protein synthesis in vivo and in vitro (Preedy et al., 1985). In a study on protein absorption, adult males were exposed to HA for a prolonged period of time (Kayser et al., 1992). Protein malabsorption did not play a role in the observed weight loss in these subjects at up to 5500 m altitude.

Patients with chronic obstructive lung disease who live at sea level are living permanently in normobaric hypoxia. These individuals have reduced digestive abilities and also a reduced ability to absorb carbohydrate (Milledge, 1972). Muscle mass also declines due to changes in protein metabolism (Morrison et al., 1988).

In contrast to mountain climbers exposed to acute hypoxia, Sherpas, resident and native to HA, do not show weight loss (Boyer and Blume, 1984). Additionally, no weight loss was observed in children of high socioeconomic level, who had normal protein intake and were born and permanently residing at HA (Post et al., 1994). However, muscle biopsies indicated a decreased muscle fiber size in HA natives and residents (Kayser et al., 1991), as has also been demonstrated in mountain climbers (Hoppeler et al., 1990).

Expeditions to Mount Everest have shown that digestibility of foods is decreased at HA. However, the altitude at which decreased carbohydrate absorption occurs is still controversial (Kayser, 1994).

Protein metabolism has primarily been studied at sea level in adults who have been exposed to HA during either a short or prolonged period of time; but studies have not been carried out in other populations such as children, those native to HA, and those permanently living at high altitude, even though approximately 140 million people around the world live at altitudes above 2500 m (WHO, 1996). In the Andean region of South America, approximately 17 million people live at altitudes ranging from 3000 to 5300 m (Pawson and Jest, 1978). This underlines the importance of understanding protein metabolism in HA residents.

The objective of this study was therefore to evaluate the effect of altitude on protein metabolism in Bolivian children. For this purpose, protein utilization was measured using leucine, which was labeled with a stable isotope (^{13}C), in two groups of children: one group born and living at high altitude (HA) and another group born and living at low altitude (LA).

MATERIALS AND METHODS

Setting

The study was carried out at two altitudes. High altitude (HA) trials were conducted at the Instituto Boliviano de Biología de Altura (IBBA) in La Paz, which is 3600 m above sea level. Low altitude (LA) trials were conducted at the Centro Nacional de Enfermedades Tropicales (CENETROP) in Santa Cruz, which is 420 m above sea level. The study was conducted in the cold season at both altitudes. Ambient temperatures were 16.5° to 18°C at HA and 22° to 27.5°C at LA. Mean values for barometric pres-

sure were 498 ± 0.6 mmHg at HA and 728 ± 1.2 mmHg at LA.

Subjects

Ten healthy male and female children (8 to 9 years old), of medium to high socioeconomic level and of mixed ancestry (Spanish and Indian), were recruited from private schools at both altitudes. The selection criteria were as follows:

- Subjects in group 1 had to be born and currently residing at HA (La Paz) and must not have traveled to LA during the last 6 months.
- Subjects in group 2 had to be born and currently residing at LA (Santa Cruz) and must not have traveled to HA during the last 6 months.
- Both groups had height-for-age and weight-for-age Z-scores higher than −1.5 (according to NCHS reference) and leukocyte counts lower than 12,000 leukocyte/mm^3 of blood. Subjects were screened for the following problems: intestinal parasitic infection, structural skeletal deformities, extended skin lesions (burns), neoplasm, congenital malformations, a history of repeated infections, prolonged systemic medication with corticosteroids, antiparasitic or any other pharmacological treatment, cytostatic and/or radiological treatment, and signs or symptoms of vitamin A or iodine deficiencies or intestinal malabsorption.

The study protocol was approved by the Technical Committee of the Instituto Boliviano de Biología de Altura. Children participated in the study only when a consent form, which described the methods to be used, was signed by the children's parents.

Anthropometry and body composition

Body weight was measured to the nearest 0.2 kg with an electronic scale (Tefal 200, France). Body height was measured to the nearest 1 mm with a specially designed meter. Upper-arm circumference was measured by means of a metric tape with an accuracy of 1 mm. Triceps skinfold thickness was measured with a caliper (Holtain Ltd., Crymych UK) to an accuracy of 0.2 mm. Hemoglobin was measured by the cyanmetahemoglobin method. Leukocytes were counted manually. The Ritchie methods was used for coproparasitological tests. Weight-for-height and height-for-age values were expressed as Z-scores according to National Center for Health Statistics reference (NCHS, WHO, 1986), using the EPI-Info 6.0 (Antrophometry, Center for Disease Control and Prevention, Atlanta, GA, USA, 1996). Body-mass index (BMI) was calculated as weight (kg)/ height (m^2) (Frisancho, 1990). Total upper-arm area (TUA), upper-arm muscle mass area (UMA), and upper-arm fat area (UFA) values were calculated using the same equation as used by Frisancho (Frisancho, 1990).

Principle of the stable isotope method

The objective was to measure leucine oxidation after a single oral dose of a protein of high biological value, in which leucine was labeled with ^{13}C in the first position.

The metabolic fate of leucine is either incorporation into protein synthesis or degradation by irreversible decarboxylation, in which $^{13}CO_2$ is produced. With this method, the total amount of expired $^{13}CO_2$ was calculated as the area under the curve of increased V_{13CO_2} over the baseline during the hours following ingestion. The difference between the amount of tracer given and the amount of tracer recovered indicates the amount of ingested protein utilized for protein synthesis. Measurements of $^{13}CO_2$ enrichments and V_{CO_2} were made for calculation of V_{13CO_2} (Beaufrérè, 1994).

The synthesis of the ^{13}C-labeled protein was made in collaboration with the Laboratoire de Technologie Laitière (J. L. Maubois, Rennes, France). Administration of the labeled protein consisted of a 24-h continuous intravenous infusion of L-(1-^{13}C)-leucine into a lactating cow, followed by collection of milk, which was then separated by ultrafiltration; finally, the two main fractions of milk protein, casein and lactoserum, were purified (Boirie et al., 1995).

Experimental design

The study lasted 7 h, during which time the children were under medical supervision. On

the day before the test, the children did not perform any intense physical activity. On the day of the test, each child arrived by car at the laboratory at 7:30 AM. Children arrived in a fasted state, having not eaten since 9:00 PM of the previous day. For the duration of the test they were resting in the laboratory at a comfortable temperature (18°C).

Measurements of leucine oxidation were performed as follows: after a short rest, two basal expired air samples were collected in vacutainers at 7:45 and 7:55 AM. At 8:00 AM an oral load of casein was given to the child. Subjects' expired gas was collected into a 100-L Douglas bag for 5 min. A gas sample was then transferred from the bag into the vacutainers (Vacutainer, ref. 6430, 10 mL, Becton Dickinson, Rutherford, NJ 07070, USA). All samples were taken in duplicate. Ventilation of carbon dioxide (V_{CO_2}) was measured for 5 min, every 30 min, during the 3-h testing period (8:00 to 11:00 AM) and every 60 min during the next 2 h (11:00 AM to 1:00 PM). Breath samples were obtained at the end of each V_{CO_2} measurement (in total 2 baselines + 8 samples = 10 samples in duplicate).

The total amount of labeled casein was dissolved in 200 mL of water and was administered at 0.5 g/kg body weight. ^{13}C leucine enrichment within the casein was 3%. The ingestion of casein was completed in an average of 5 min.

The content of carbon dioxide and of oxygen in expired air was measured with a Gould Godart Capnograph Mark III and Servomex A570, respectively. The total expired volume was measured using a 100-L spirometer (Tissot). The $^{13}CO_2$ content of the expired air in the vacutainers was measured in the Human Nutrition Laboratory in Clermont-Ferrand, France (Microgas + Optima IRMS, VG instruments) by mass spectrometry.

Calculations

The amount of $^{13}CO_2$ produced from oxidation of the ingested leucine (μmol/kg/min) was calculated using the following equation:

$$F^{13}CO_2 = \frac{F_{CO_2} \times E_{CO_2} \times 44.6}{\text{wt (kg)} \quad 100 \times K}$$

where F_{CO_2} is the proportion of CO_2 produced (mL/min), ECO_2 is the $^{13}CO_2$ enrichment in expired air (atom percent excess), and wt is the body weight of the subject (kg). The constant 44.6 mol/mL was used to convert F_{CO_2} into micromoles per minute at standard temperature and pressure. The factor 100 changes atom percent excess from a percentage value to a fraction. Factor K (where $K = 0.8$) corrects for the fraction of $^{13}CO_2$ released by (1-^{13}C) leucine oxidation, but not recovered in expired air.

Statistical analysis

All results are expressed as means ± SD. The statistical analysis of data sets was performed using EPI-Info 6.0 (Center of Diseases Control and Prevention, Atlanta, GA, USA), STATISTIX 4.1. (Analytical Software, P.O. Box 12185, Tallahassee, FL, USA). Statistical differences between the two groups were assessed using a two sample t-test.

RESULTS

Two of the ten children included in the study at altitude (group 1) were excluded from analysis. One subject was excluded for vomiting the casein solution, and the second subject was excluded because he was holding his breath during the expired air collection. Three subjects were excluded from the LA trial (group 2) for holding their breath during the expired air collection and one for technical problems during the test.

Table 1 presents the nutritional characteristics of the 14 children who participated in the study. Mean height-for-age and weight-for-height scores were not different between groups. Moreover, no child had height-for-age or weight-for-height Z-scores below -1.5 or higher than 1. Mean BMI was not different between groups, and no child had a BMI lower than the 10th percentile or higher than the 90th percentile (Frisancho, 1990). Total upper-arm area (TUA) and upper-arm muscle mass area (UMA) were not significantly different between groups. However, the arm fat index (AFI) was higher in group 1. Group 1 had a significantly higher hemoglobin concentration

TABLE 1. ANTHROPOMETRY AND HEMATOLOGY

	Group 1 (HA) (n = 8)	*Group 2 (LA) (n = 6)*	p[a]
Age (months)	105.07 ± 6.0	107.2 ± 8.0	0.57
Weight (kg)	27.8 ± 3.2	27.9 ± 4.5	0.97
Height (cm)	131.4 ± 6.7	128.6 ± 5.8	0.43
Weight-for-height (Z-score)	−0.07 ± 0.82	0.31 ± 0.69	0.37
Height-for-age (Z-score)	0.09 ± 1.17	−0.57 ± 0.53	0.23
BMI weight (kg)/height (m^2)[b]	16.1 ± 1.08	16.7 ± 1.68	0.39
TUA (cm^2)[c]	18.6 ± 1.3	18.1 ± 1.9	0.50
UMA (cm^2)[d]	19.3 ± 2.3	19.7 ± 4.5	0.80
AFI (%)[e]	30.0 ± 3.5	24.9 ± 3.0	0.01
Hemoglobin (g/L)	150.5 ± 0.5	121.3 ± 0.5	0.00001
Leukocyte count (mm^3)	6453 ± 2389	7566 ± 1506	0.33

Values are means ± SD; *n* = no. of subjects.
[a]Student's *t*-test.
[b]BMI, body mass index.
[c]TUA, total upper-arm area.
[d]UMA, upper-arm muscle mass area.
[e]AFI, arm fat index.

when compared with group 2. This result was to be expected in children living permanently at HA. Conversely, leukocyte count was not different between groups.

Quantity of ingested casein and duration of ingestion were not significantly different between groups (Table 2). The mean production of $^{13}CO_2$ over the 300 min following casein ingestion was higher in group 2 than in group 1 (0.008 and 0.006 μm/kg/min, respectively). This difference was apparent 30 min after ingestion and lasted for the total duration of the study (Fig. 1).

When expressing leucine oxidation as a fraction of the ingested leucine, more of the ingested leucine was oxidized by the children living at LA (25.2%) than by the children living at HA (19.7%) (Table 2, Figs. 1 and 2).

The average elimination of $^{13}CO_2$, expressed in μmol/kg/min during the 300 min of the test (Table 2) was significantly higher in group 2 ($p = 0.04$).

Table 3 describes oxygen consumption, carbon dioxide elimination, and the respiratory quotient. V_{O_2} and V_{CO_2} were higher at HA than LA, and difference in V_{O_2} was significant ($p = 0.05$).

Some foods, such as popcorn, cane sugar, millet, and polenta, naturally contain higher concentrations of ^{13}C. The diets of both groups were assessed, and no significant difference was found in their consumption of these natu-

TABLE 2. UTILIZATION OF CASEIN

	Group 1 (HA) (n = 8)	*Group 2 (LA) (n = 6)*	p[a]
Casein ingested (g)	13.8 ± 1.5	13.6 ± 2.3	0.84
Ingestion time (min)	4.93 ± 1.8	3.16 ± 2.9	0.19
Isotope dosage:[b]			
$^{13}CO_2$ (μmol/kg/min)[c]	0.00597 ± 0.0054	0.00804 ± 0.0057	0.04
$^{13}CO_2$ (initial 30 min)[c]	0.288 ± 0.18	0.781 ± 0.44	0.01
$^{13}CO_2$ cumulative (%)[d]	19.7 ± 4.9	25.2 ± 3.2	0.02

Values are means ± SD; *n* = no. of subjects.
[a]Student's *t*-test.
[b]Isotope dosage by expired air content $^{13}CO_2$.
[c]Leucine oxidation.
[d]Cumulative leucine oxidation at 300 min postingestion.

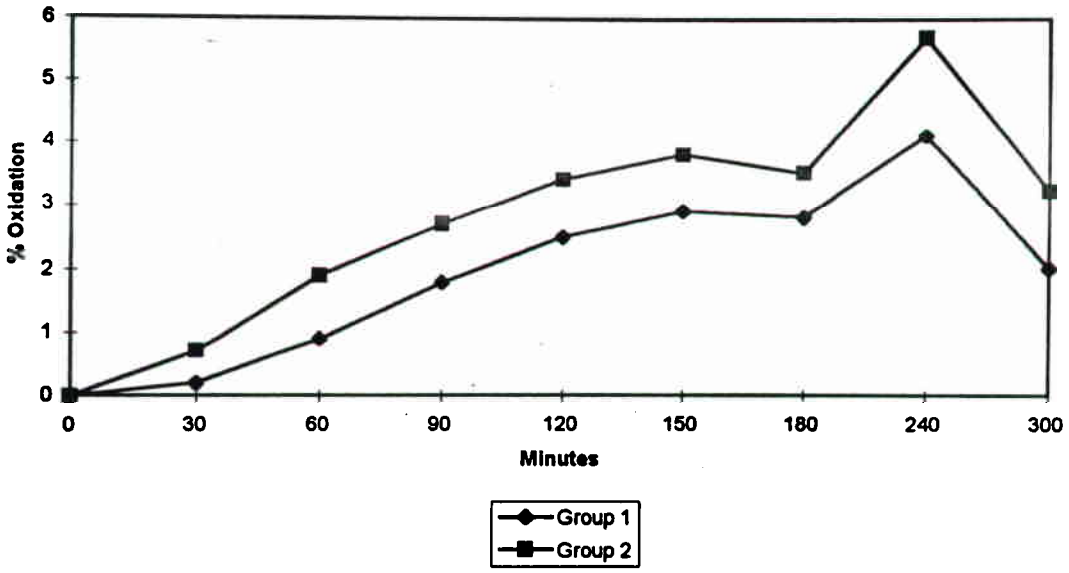

FIG. 1. Mean percentage (1-^{13}C) Leucine oxidation in children resident at high altitude (group 1) and low altitude (group 2).

rally ^{13}C enriched foods. Finally, in the HA group, 19.7% of the ingested tracer (^{13}C-leucine) was recovered in expired air during the 300 min after casein ingestion, which means that 80.3% of the ^{13}C-labeled leucine was utilized. On the other hand, at LA 25% was recovered at 300 min and 75% of the ^{13}C-labeled leucine was utilized.

DISCUSSION

Protein metabolism, assessed by leucine labeled with a stable isotope (^{13}C), was affected by HA in school children native to and permanently living at HA (3600 m). We found that the appearance of labeled CO_2 in the expired air was lower in HA than in LA children after

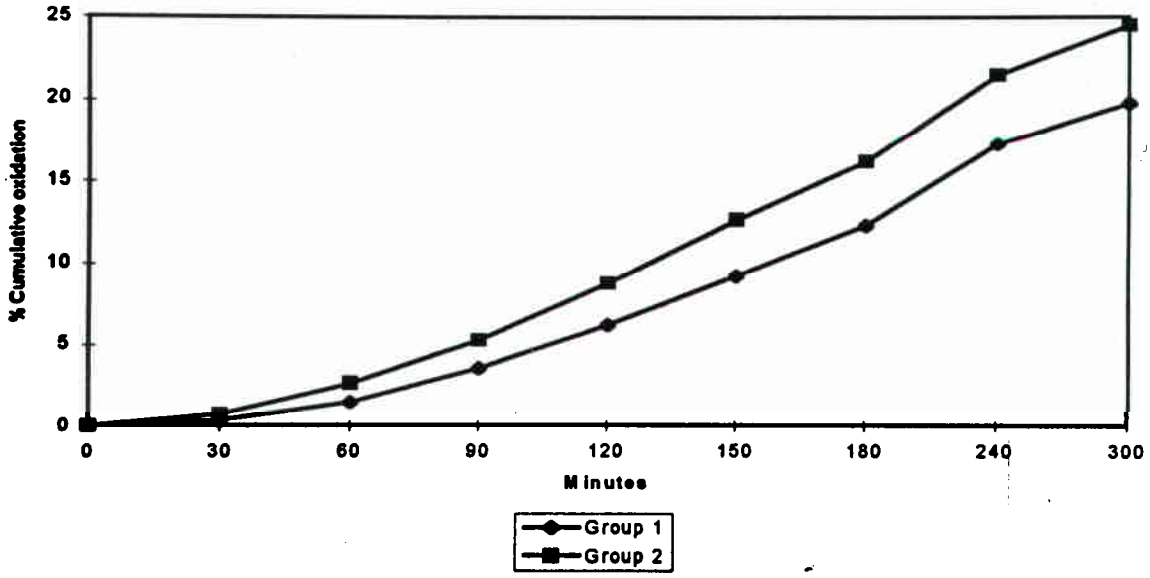

FIG. 2. Mean percentage cumulative (1-^{13}C)-leucine oxidation in children resident at high altitude (group 1) and low altitude (group 2).

TABLE 3. ELIMINATION OF CO_2 AND O_2 CONSUMPTION

	Group 1 (HA) (n = 8)	*Group 2 (LA) (n = 6)*	p[a]
VCO_2 (mL/min)[b]	162.9 ± 23.9	144.5 ± 20.4	0.15
VO_2 (mL/min)[c]	183.9 ± 34	153.3 ± 6	0.05
QR[d]	0.893 ± 0.1	0.941 ± 0.1	0.42
^{13}C enrichment (μmol)	306.14 ± 34.9	281.19 ± 48.1	0.28

Values are means ± SD; *n* = no. of subjects.
[a]Student's *t*-test.
[b]Elimination of carbon dioxide.
[c]Oxygen consumption.
[d]Respiratory quotient.

having ingested the same amount of tracer. There are two possible explanations. First, less leucine may have been absorbed at HA and therefore less was oxidized and less would appear in expired air. The second possibility is that the same amount of leucine is absorbed at HA and LA, but that leucine oxidation was impaired at HA and therefore more of the ingested leucine was used for protein synthesis. However, protein synthesis uses leucine from the diet (here the casein) and also from endogenous protein degradation. The methods used in this study did not allow endogenous protein degradation to be measured. Therefore, if we consider that the second hypothesis is correct, we can only say that a fraction of protein synthesis, and not "protein synthesis" as a whole, is increased in HA children. Reduced absorption is certainly possible and also should be considered.

We were unable to identify which of these two hypotheses was responsible for our results because only a single oral tracer was used. An intravenous tracer would be necessary to make this distinction. However, field constraints forbid the use of such a tracer in our study. For these reasons, this paper shows that protein absorption and/or utilization is affected by altitude in children.

In any case, our results are similar to those of Fujita (Fujita et al., 1982), who studied the highlanders from Papua New Guinea at 1500 m above sea level. These people utilized proteins in a more efficient way than Japanese subjects. The highlanders ingested essentially the same quantity of proteins, but had a lower urinary loss of endogenous nitrogen. These findings suggest that protein metabolism is different at altitude (Fujita et al., 1984). Another study was performed in rats that were acclimatized to HA for many generations. It was estimated that HA produced an elevated metabolic rate during growth, suggesting that at HA the metabolic demand is increased (personal communication, Joseph V., 2000).

Hemoglobin concentration was higher in children living in La Paz than in children living in Santa Cruz according to the appropriate cutoff value of hemoglobin concentration for the altitude of the setting (Berger et al., 1994, 1996, 1997a; Yip, 1993). None of the children were anemic. Higher hemoglobin concentration in children living at high altitude is caused by the lower partial oxygen pressure at HA. Indeed, Hurtado et al. demonstrated that people living at HA have an increased number of erythrocytes and a higher hemoglobin concentration due to an adaptive phenomenon to maintain an adequate oxygen transport capacity (Hurtado et al., 1945). These findings were confirmed in more recent studies conducted in children (Estrella et al., 1987; Freire, 1989; Berger et al., 1994, 1997a; Dirren et al., 1994) and in adults (Berger et al., 1997b) living in the Andean region. This increase in protein, as hemoglobin, could be evidence that points in the direction of an increase in protein synthesis in people native and permanently living at HA. The increase of V_{O_2} and V_{CO_2} in the HA children indicates a higher resting metabolic rate (RMR). This finding is in agreement with previous measurements of basal metabolic rate (BMR) at altitude, as briefly described by Ward et al. (2000). Mawson et al. (2000) have found

a transient increase of 6.9% in BMR during acute exposure of women to an altitude of 4300 m. Our finding of an increased resting metabolic rate at HA could at least in part explain the higher protein turnover in the HA children.

The results of the present study provide new knowledge concerning protein metabolism in children living at HA. However, the study of amino acid kinetics with isotopic methods is generally only conducted 3 to 4 h postingestion (Beaufrérè et al., 1989), and not during the first minutes postingestion. This study demonstrated that $^{13}CO_2$ elimination is decreased at HA from soon after ingestion, which suggests that HA may cause a faster and greater protein utilization and/or a minor increase in protein absorption. Moreover, the accumulated elimination of $^{13}CO_2$ was also different between groups from the beginning of the follow-up period onward, and the difference increased with time.

Stable isotopic tracers were used, and this method may present some limitations. The first limitation concerns the administration of isolated labeled amino acids not bound to a protein. Under normal conditions, complete proteins are ingested, and their digestion and absorption differ from the absorption of isolated amino acids (Beaufrérè, 1989). Second, in some studies the administration of amino acids was continuous or fractionated every 15 to 30 min (i.e., through a nasogastric catheter). This method is not representative of normal meal intake, which typically occurs every 4 to 6 h (Motil et al., 1981). The fractionated or continuous administration would stimulate the neuroendocrinous regulation of the digestive system in an unphysiological manner (Beaufrérè et al., 1991; Guyton, 1997). To overcome these limitations in our study, the casein was offered in one single ingestion, a situation that more closely simulates normal daily food intake.

Therefore, the stable isotope tracer methodology used here, which is in accordance with ethical rules (Beaufrérè, 1991), improves our knowledge of protein metabolism in children living at high altitude.

CONCLUSION

This study demonstrated the current feasibility and accuracy of using a synthetic protein of high quality labeled with a ^{13}C stable isotope by a noninvasive method to measure protein metabolism in children living in developing countries.

Protein utilization and/or absorption was affected by HA in schoolchildren native to and living at high altitude. These results improve the understanding of possible adaptation to protein metabolism that occurs in children living at high altitude.

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Address reprint requests to:
José Luis San Miguel, M.D.
Instituto Boliviano de Biología de Altura
Facultad de Medicina
Universidad Mayor de San Andrés
Calle Claudio Sanjinez S #. Miraflores
La Paz, Bolivia
P.O. Box: 641
Telephone: 2242059 - 2242064
Fax: 2361419

E-mail: josanto10@yahoo.es

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