EFFECT OF ALTITUDE ON THE PROTEIN METABOLISM OF BOLIVIAN CHILDREN

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Abstract

The malnutrition is prevalent and is a major problem among Bolivian children. It is caused by several interacting factors: (i) inadequate protein energy intake due to low socio-economic status; (ii) exposure to acute, repeated and chronic bacterial infections; (iii) exposure to multiple and chronic parasitic infections; (iv) high altitude of the capital, La Paz, 3600 m, with a numerous populations compared to the rest of the country. The research objectives in the first phase are: (i) determination of protein utilization with a non-invasive method using stable isotope tracer among children living at high and low altitude; (ii) determination of protein metabolism among eutrophic children without parasitic or acute bacterial infections at both altitudes; (iii) determination of protein requirement among these children. Two groups of 10 pubertal children, matched for age and sex, of same socio-economic status, eutrophic, without malnutrition, infections or intestinal parasites will be studied; the different status being arrived by anthropometric, nutritional intake, biochemical and pediatrical evaluation. For the metabolic study, stable isotopes L-[1-13C] leucine labelled casein will be used and $^{13}CO_2$ excreted will be measured. All the basic nutritional assessment and VCO_2 measurements will be performed in Bolivia, while the samples of expired gas will be stored in Vacutainers for further analysis by isotope radio mass spectrometer (IRMS), in Clermont-Ferrand, France. The plans for future work is based on the study of the effects of the different variables and their interactions. The following will be evaluated: (i) the socio-economic status; (ii) the bacterial infections: (iii) the parasitic infections; (iv) the altitude. As published by Obert, et al., the socio-economic variable is more connected with the nutritional status than with the altitude.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Malnutrition is a prevalent problem in Bolivian children. It might result from the interactive effects of at least four factors which are (i) inadequate protein energy intake due to a low socio-economic status; (ii) chronic and/or frequent acute bacterial infections; (iii) chronic multiple parasitic infections and; (iv) living at high altitude since La Paz, the capital of Bolivia is located at 3600 m above sea level, with the biggest population compared to the rest of the country.

High altitude can interfere with nutrition and growth in humans by four mechanisms: (i) reduced total food intake; (ii) impaired nutrient bioavailability; (iii) imbalance between an increased energy expenditure and a reduce energy intake and; (iv) loss of muscle mass.

Anorexia is a common problem in alpinists and during acclimatization to high altitude [1,2]. This problem is unlikely to be of importance in natives of high altitude, and actually, food intake in Bolivian children of high socio-economic status living in La Paz are normal or possibly elevated [3,4,5].

Energy balance is difficult to maintain above 4500 m, partly due to an increased resting metabolic rate and partly to lowered energy intake [1]. This imbalance does not seem to exist in Bolivian children of high socio-economic status [3]. Food digestibility might be impaired at high altitude as was reported during a Mount Everest expedition [2]. A decreased xylose absorption was also noted in hypoxic sea level patients. There exists controversy about the altitude at which such a problem might occur (4000m ?, 6000m ?) and it is usually considered that the problem is of limited importance unless an extreme altitude is reached. However, to our knowledge, this point was never considered in children living permanently at high altitude. It is noticeable that, in a recent food inquiry in La Paz by G. Post and P. Obert [4,6], the mean protein intake of girls of high socio-economic status was 93 ± 27 g/day. The girls were 11.3 ± 0.6 years old and were not obese. Such a protein intake is quite high (3 g/kg/day) which might suggest that a part of this intake is not absorbed. Another study shows that boys with low socio-economic status at low and high altitude have significantly lower pre-albumin levels than boys with high socio-economic status [7].

In another study at high altitude (3600m and 4800m) in Bolivia, in children of low socio-economic status the authors report that the total energy input was very low and covered only 60% of the energy requirement, the quality of the ingested proteins being very poor [8].

A direct effect of hypoxia on protein metabolism and a specific activity on muscle is possible [9]. A rapid muscle loss is common in alpinist [1]. In addition to the factors listed above which could contribute to muscle loss, acute hypoxia was also shown to reduce leucine turnover and uptake of leucine from the forearm [10], and chronic normobaric hypoxia also results in similar changes in protein metabolism [11]. Acute hypoxia was shown to decrease protein synthesis in rat tissues in vitro and in vivo [12].

We proposed a first phase to study the effects of independent variables (altitude - inadequate protein energy intake - bacterial infections - parasitic infections). The later stage studies would be on the interactions. For practical reasons, the first variable to be studied will be altitude, which is certainly the most specific characteristic of Bolivia, in comparison to other developing countries in which malnutrition is common.

In summary, possible decreased digestibility and a direct effect of hypoxia on protein metabolism will be the variables tested in the following protocol.

1.1. Research objectives in the first phase

Main Objective:

To asses the effect of altitude on absorption and use of protein through stable isotopic tracers on wealthy children.

Specific Objectives:

- Determination the effect of altitude on absorption and use of protein in eutrophic children without parasitic or acute bacterial infections to be compared with children of same characteristics residing at sea level through stable isotopic tracer 13C-Leucine.
- Determination of protein requirement in these children.

1.2. Collaboration with other research groups

The Department of Nutrition from the beginning works together with The French

Institute of Scientific Research for the Development in Cooperation O.R.S.T.O.M., whose center is in Montpellier, France. Initially, research was carried out in the following fields :

a. Nutritional immunology, especially in the severely malnourished child, in integrated rehabilitation using thymus echography as a risk indicator in order to release the child based on immunological, clinical and nutritional criteria and thus avoiding pseudo-recovery.

b. Haematological indicators at high altitude. This work was finished, which permit to define vulnerable population groups, children and women in the reproductive age. Thresholds of nutritional anemia at altitude were investigated during a follow-up study with a double-blind essay of an iron-folate vs. placebo supplementation. The values for haemoglobin were found to be significantly higher than recommended by the WHO. These results may have a great impact on public health for these populations in assessing prevalence of anaemia and to plan interventions.

c. Growth retardation and evaluation of child development. This study was in the last phase.

2. METHODS

2.1. Parents consent

For ethical reasons consent will be obtained from the parents stating principally the atraumatic, non-invasive method to be used. Only when the consent is obtained, the child will be evaluated. (See Annexes 1 & 2).

2.2. Subjects

Two groups of 10 Bolivian children will be studied, one living at high altitude, La Paz 3600m, and one at low altitude, Santa Cruz 420m . The groups will consist of pre-school children, age 8 to 9 years old, matched for age and sex ratio, of high socio-economic status.

2.3. Criteria for inclusion

- Natives and permanent residents at high altitude (La Paz).
- Natives and permanent residents at low altitude (Santa Cruz).
- Normal healthy children for the anthropometric indicators: weight/age, height/age and weight/height which will be below +2 SD and above -1 SD compared to NCHS standards for each parameter.
- Normal physical examination including thymus echography carried out by a paediatrician.
- Normal biochemical, nutritional indicators:
    - Albumin
    - Prealbumin
- Absence of acute infection:
    - White blood cell count
    - C - reactive protein
    - Orosomucoid
- Absence of parasitic infection in 3 consecutive stool tests, and test Graham.

2.4. Criteria for exclusion

- Structural skeletal deformities and extended skin lesions (burns)
- Auto-immune diseases, neoplasm
- Congenital malformation
- Primary or acquired immunodeficiencies
- History of repeated infections
- Prolonged systemic medications with corticosteroids
- Antiparasitic treatment, or any other pharmacological treatment
- Cytostatic and/or radiological treatment
- Staying at low altitude during the last 6 months
- Specific nutritional deficiencies
- Symptoms or signs of intestinal malabsorption

The method will have to be used under field conditions in children and thus has to be non-invasive. It is a measurement of whole body total leucine oxidation after the ingestion of an oral load of L-[1-13C] leucine labelled milk protein (casein).

Such protein is obtained by continuous intravenous infusion of a cow for 24 hours, the milk being collected both during and after the tracer infusion. Proteins are then purified from the milk by ultrafiltration and lyophilized. Exogenous leucine oxidation is measured in expired CO_2 (as the product of VCO_2 by ^{13}C-CO_2 enrichment measured by IRMS). Total leucine oxidation is calculated as the area under the curve of $V^{13}CO_2$ increase above baseline. Preliminary studies in 6 normal adult volunteers in France have shown that: (i) $^{13}CO_2$ has returned to baseline 5 hours after the load (which means little or no tracer recycling); (ii) peak $^{13}CO_2$ occurs at 100-160 min and; (iii) 30 ± 1.5% (mean ± SD) of the ingested tracer is recovered after 6 hours, suggesting that 70% of the absorbed leucine was retained for protein synthesis. A classic 80% CO_2 recovery was used for calculation which might be an underestimate. It should also be kept in mind that these methods measure only exogenous leucine oxidation. The results were obtained with lactoserum protein and might be different with casein, at least in terms of kinetics, since casein is more slowly absorbed. Finally, the shape of the curve might be an indication of the efficiency of protein digestion and absorption. Preliminary data in patients with moderate pancreatic insufficiency show a much slower appearance of $^{13}CO_2$ in the breath.

Measurements of leucine oxidation will be performed as follows:

- It will be done after a brief fast (post absorptive state, *i.e.* at 8:00 am after a normal evening dinner).

- Collection of breath samples in Vacutainers at 7:45 and 7:55 a.m. All samples will be taken in duplicate. The child will have to breeze in a Douglas bag for 5 minutes, a gas sample being then transferred in the vacutainers from the bag.

- At 8:00 am, ingestion (in 5 minutes) of 0.5 g/Kg of labelled protein dissolved in water (200 ml). The protein will be casein, labelling is approximately 3.5%. The dose will be accurately weighed.

- VCO_2 will be measured during 5 min., every 30 min. during 3 hours (8:00 am -11:00 am) and every 60 min. during the next 3 hours (11:00 am - 14:00 pm).

- Breath samples will be obtained at the end of each VCO_2 measurement (total : 2 baseline + 9 samples = 11 samples in duplicated) and collected in Vacutainers.

- Vacutainers will be sent to the Human Nutrition Laboratory in Clermont-Ferrand, France, for $^{13}CO_2$ analysis (Microgas + Optima IRMS, VG instruments).

3. RESULTS

3.1. Results of the pilot experiment

Once the 4 trials have taken place with 4 children we think we have developed a suitable technique to collect expired air avoiding CO_2 "contamination". The last curve shows suitable results. See Appendix 3.

We believe (no inferential estimation) that similarly to the experience with 6 elder volunteers, a curve of % leucine oxidation at 3600m altitude shows the following profile.

- $V^{13}CO_2$ goes back to baseline 5 hours after charge
- $^{13}CO_2$ maximal value appears between 90 and 150 min.
- 34.44% of ingested trace element (tracer) was recovered after 6 hours which means 65.56% of labelled leucine was absorbed and used for protein building up.

4. PLANS FOR FUTURE WORK

During the 2nd phase the interaction of the aforementioned variables will be assessed. If the hypothesis of phase 1 as demonstrated to be true, *i.e.* if high altitude is not a determinant factor for protein metabolism, then during the second phase we will proceed as follows.

A random sample of 40 children, age 8-9 years from La Paz, will be divided in 2 groups, one group consisting of malnourished and the other of well nourished children matched for age and sex. Both groups will be divided into 2 sub-groups, one with parasitic infection and one without.

The objectives will be as follows:

- Evaluation of protein utilization with a non-invasive method using stable isotope tracer among undernourished children with or without parasitic or acute bacterial infections versus control children (eutrophics) without infections.

- Determination of protein requirement among these children.

Institutions like WHO, FAO,UNU, and others continue to search for a method to assess the real protein and energy requirement in developing countries, principally in the children of these countries.

Energy expenditure is given by several variables like resting metabolic rate, the thermic effect of food, exercise, thermogenesis. Which of these variables contains the energy expenditure which carries the immune defense of the organism?

Children in developing countries are constantly exposed to bacterial, viral and parasitic infections. How much food do these children really need in order to defend themselves against constant stress?

Beisel (1977) has defined the nutritional response to an infection how does one recover from the whole negative balance which is produced during the natural history of an infection? Infection and disease can be evaluated clinically and through laboratory testing. However, the immune system together with other systems control and equilibrates constantly the organism from aggressions like infections, *i.e.* through intercellular cooperation, molecular homeostasis can be produced in order to stop, destroy pathogenous agents without affecting the rest of the organism. Are these the so-called inapparent, benign infections? How much energy will the immune system consume in these situations?

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"FICHA DE INFORMACION ENTREGADA AL RESPONSABLE LEGAL DEL NIÑO HA INCLUIRSE EN EL ESTUDIO"

ESTUDIO DEL EFECTO DE LA ALTURA SOBRE EL METABOLISMO PROTEINO EN NIÑOS BOLIVIANOS

Las proteinas son uno de los componentes mayores del organismo y estan particularmente presentes dentro del músculo, el hígado y el intestino. Estas proteinas estan en permanente construcción, es decir en constante síntesis, y también en permanente destrucción o degradación. Un buen estado nutricional depende del equilibrio entre la síntesis y la degradación. Los seres humanos comemos alrededor de 100 g de proteina por día, sea de la carne, la leche,....., y estas proteinas alimenticias constituyen diariamente las proteinas del organismo.

El objetivo de esta investigación es :

I. Comprender mejor como una proteina alimenticia es utilizada por el organismo para participar en el equilibrio nutricional en niños de 8 a 9 años de edad.

2. Verificar cual es el efecto de la altura sobre la utilización de las proteinas en niños de 8-9 años de edad.

Los beneficios que se obtendran de esta investigación son dirigidos a los niños que viven a gran altura, ya que se aportará información que permita conocer sobre la absorción y utilización de la proteina animal y dara probablemente información sobre las necesidades de proteina de un niño habitante de gran altura para mantener su salud.

Los isótopos estables son "marcadores" como 13C-Leucina que es un aminoácido componente de una proteina, como en nuestro caso la caseína de la leche de vaca y que permitirá estudiar la utilización de la proteina una vez que es administrada por vía oral. Su inocuidad, es decir su característica de no ser dañina para la salud en seres humanos esta ampliamente demostrada por numerosos trabahos científicos publicados internacionalmente. Este trabajo es parte de un conjunto de investigaciones que se llevan a cabo actualmente en varios países del mundo en los que se usan isótopos estables y donde el sujeto de estudo son niños de diferentes edades, desde lactantes hasta escolares. La eficacia de los isótopos estables en diferentes metodologías esta fehacientemente demostrada.

Se mantendrá la confidencialidad de los registros obtenidos de cada niño mediante sistema de claves en un computador donde solo tendran acceso los investigadores del proyecto y eventualmente previa autorización personal técnico.

La realización de este Protocolo de Investigación necesita que se demuestre que el niño esta sano y normal al momento del estudio. Para ello se realizará pocos días antes:

1. Historia Clínica Pediátrica, con un interrogatorio y un examen físico pediátrico
2. Antropometría nutricional
3. Ecografía de Timo.
4. Bioquímica sanguínea con dosificación de: Hemoglobina (se necesita 20 microlitros de sangre). Hematocrito, número de Globulos Rojos y de Globulos Blancos (se necesita 50 microlitros de sangre). Marcadores Bioquímicos Nutricionales: albúmina, prealbúmina, orosomucoide, proteina C reactiva (se necesita 200 microlitros de sangre). para ello se realizará una punción capilar en

un dedo, para obtener un total aproximado de 10 gotas de sangre que equivalen más o menos a los 270 microlitros de sangre especificados anteriormente.

5. Parasitología intestinal: se realizará un estudio coproparasitológico seriado de 3 días seguidos y un test de Graham para cada niño.

La normalidad de estos resultados más el examen clínico pediátrico permitirán establecer que el niño se encuentra sano al momento, pudiendo incluírselo en el estudio.

El niño debe permanecer en el Instituto Bolviano de Biología de Altura un total de 7 horas, desde 7:30 a m hasta 2:30 pm del mismo día de estudio. Previamente debe haber visitado el instituto para conocer al personal, las instalaciones y el material que se usara en la prueba. También, a modo de entrenamiento se le indicaran las fases de la prueba.

Es necesario que el niño se encuentre en ayuno desde la noche anterior, habiendo ingerido su cena habitual. En esas condiciones ingresara al instituto y se iniciará la prueba con la recolección de aire espirado por el niño durante 5 minutos en una bolsa de Douglas, la misma se repetirá a los 10 minutos.

Posteriormente se realizará la ingestión de la proteina de leche, la caseína, marcada con 13C-Leucina, que es un aminoácido y a su vez componente natural de las proteinas que se encuentran dentro del organismo. A partir de este momento y durante las 3 horas siguientes se realizará la toma de aire espirado como se ha indicado anteriormente cada 30 minutos, hasta horas 11:00 am. Las 3 últimas horas se tomaran muestras de aire espirado cada 60 minutos hasta horas 14:00.

Como se ha descrito, el niño recibirá un alimento que es la proteina de leche de vaca, llamada caseína, ella será absorbida y utilizada como alimento en su organismo durante las 7 horas, podrá consumir agua durante el estudio.

Durante el test solo se pueden realizar movimientos livianos, se puede permanecer sentado, echado, caminar muy poco, lo necesario, podrá ver televisión con diferentes videos adecuados a su edad y gusto, leer, escuchar radio o jugar con material propio.

El estudio es atraumático, ya que la evaluación previa de su estado de salud solo requiere un "pinchazo" en el dedo de su mano y, la extracción de 10 gotas de sangre, ello conlleva una posibilidad mínima de infección en la zona de punción, situación que no es relevante debido a las medidas de asepsia y antisepsia necesarias que son tomadas para evitar estos problemas. En todo los momentos del estudio permanecerá a su lado un médico que cuidará su integridad junto al acompañante del niño. El producto administrado no tiene ningún efecto nocivo conocido. Existiendo innumerables estudios en seres humanos con estos productos.

Este mismo protocolo se lo llevará a cabo en una zona de baja altura, como lo es Santa Cruz, así se podrá verificar si existe un efecto de la altura en el metabolismo proteico en niños.

Anexo 2

ACUERDO DE PARTICIPACION EN EL ESTUDIO:

EFECTO DE LA ALTURA SOBRE EL METABOLISNO PROTEICO EN NIÑOS BOLIVIANOS

Investigador Principal : Dr. J. L. SAN MIGUEL SIMBRON

Yo, el firmante de este
documento,.....
Nacido el..... y domiciliado
en.....
Autorizo y estoy de acuerdo en forma voluntaria en la participación de mi hijo/a en este estudio, para lo cual estoy legalmente apto. Declaro lo siguiente :

- que el Doctor..... me ha propuesto la participación de mi hijo/a en el estudio arriba mencionado.

- que el me ha hecho conocer en forma clara:

  * el objetivo, el método y la duración del estudio
  * las molestias y los riesgos potenciales a que expongo a mi hijo/a.
  * los beneficios que aportara este estudio.
  * el conocimiento y el aviso de un Comitó Consultativo de Protección de las Personas.
  * el derecho de mi hijo/a a rehusarse de participar y en caso el acuerdo de retirar mi consentimiento en cualquier momento sin tener ninguna responsabilidad al respecto.
  * el me ha explicado en detalle el protocolo
  * respondere con veracidad en todo momento a las preguntas concernientes al estado de salud de mi hijo/a y sobre su participación en otros estudios.

Después de haber discutido libremente y obtenido respuesta a todas mis preguntas, yo acepto como responsable legal de mi hijo/a estar en todo conocimiento de causa para hacerlo participar en este estudio.

Este documento puede estar firmado por uno o ambos padres, para cuyo caso se mantiene el conocimieto y la aceptación de ambos.

Realizado en.....
en fecha,.....

Firma con aclaración

No. de C.I.:.....

Consentimiento obtenido or el Doctor:

TABLE I. BREATH TEST AFTER INGESTION OF AN ORAL DOSE OF L-[1-^{13}C] LEUCINE-LABELLED CASEIN

Child 2 bis age- 9.29; poids - 30.2; Delta CO_2 basal - 21.36;
dose ^{13}CLeu (μmoles) - 217.19); total Leu (μmoles) - 6417

Temps (minutes)	DELTA CO_2 delta 0/00	VCO_2 ml/mn	$V_{13}CO_2$ μmoles/kg. min	$V_{13}CO_2$ intégré μmol/kg	% dose oxydé	Cumul % dose oxydé
0	-21.36	185	0.000	0.000	0.000	0.00
30	-20.57	192	0.003	0.046	0.642	0.64
60	-18.88	202	0.010	0.199	2.764	3.41
90	-17.29	212	0.018	0.415	5.775	9.18
120	-17.57	199	0.015	0.492	6.848	16.03
150	-17.73	143	0.011	0.388	5.392	21.42
180	-18.13	170	0.011	0.325	4.524	25.95
240	-20	167	0.005	0.473	6.574	32.52
300	-21.36	183	0.000	0.138	1.924	34.44
360	-21.36	167	0.000	0.000	0.000	34.44

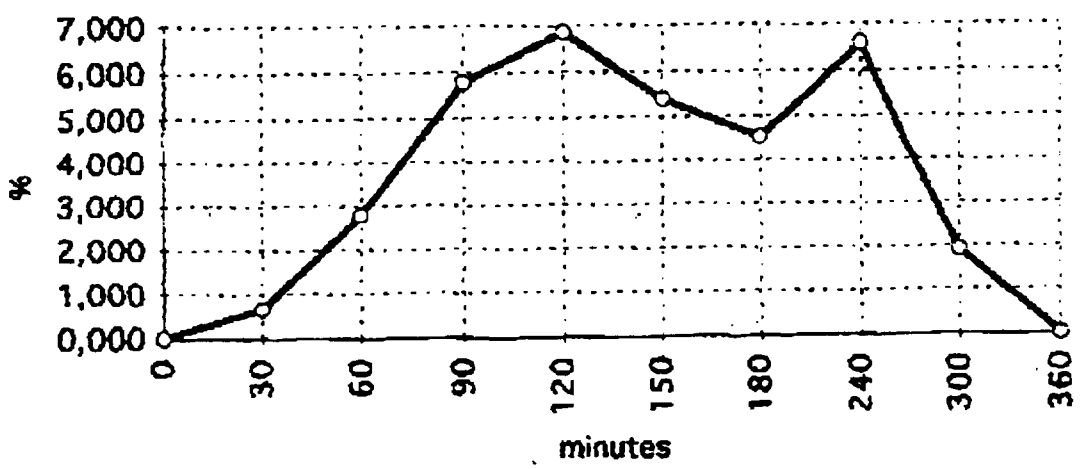

Figure 1. Percentage of the dose of L-[1-^{13}C]leucine oxidized