

# Definition and Prevalence of Anemia in Bolivian Women of Childbearing Age Living at High Altitudes: The Effect of Iron-Folate Supplementation

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*This paper discusses the effect that high altitudes have on iron metabolism and summarizes the results of an iron-folate supplementation trial. The two main objectives of the trial were to determine hemoglobin cut-off values for the diagnosis of anemia in Bolivian women of childbearing age living at high altitudes, and to estimate the prevalence of anemia in this population. The study showed that nutritional anemia is an important public health problem in such populations and that many methods of assessing it lead to an underestimation of prevalence. The cut-off values defined through this study, one of the few iron supplementation trials conducted at high altitudes, confirm the need to establish revised hemoglobin values for the diagnosis of anemia in populations living at high altitudes.*

## Introduction

It is estimated that approximately 1.3 billion individuals in the world suffer from anemia,<sup>1</sup> making it one of the most important public health issues on the international agenda. In developing countries, iron deficiency afflicts some 2 billion people and is the principal cause of anemia.<sup>2</sup> Women of childbearing age along with children and pregnant women constitute the group most vulnerable to iron deficiency anemia. The knowledge of the prevalence of anemia in these groups is fundamental for the planning and execution of effective interventions by health authorities.

The prevalence of anemia within a population depends on the criteria chosen for its definition. The measure of concentration of hemoglobin is the laboratory test most commonly used for the detection of anemia in clinical and public health studies. Because the majority of anemias in

children and women of childbearing age are linked to iron deficiency, the principal objective for the diagnosis of anemia is to detect individuals at high risk of deficiency of this micronutrient.

The diagnosis of anemia requires not only appropriate analytical methods but also cut-off values for the concentration of hemoglobin useful for the definition of anemia. The World Health Organization (WHO) and the International Nutritional Anemia Consultative Group (INACG) have defined the reference values of hemoglobin concentration to define anemia considering age, sex, and certain physiologic circumstances such as pregnancy.<sup>3,4</sup>

On the international level, these proposed reference values have been established on the basis of studies done on populations living at sea level. However, adaptation to living at high altitudes carries an increased blood capacity for the transportation of oxygen. Persons living at high altitudes have higher concentrations of hemoglobin and hematocrit than do those living at sea level.<sup>5-7</sup> This variation is due to the decrease in the partial pressure of oxygen at high altitudes, which induces a decrease in the absolute rate of oxygen available per unit of pulmonary surface<sup>8</sup> and a reduction in the saturation of oxygen in the blood.<sup>5</sup>

The response to this hypobarometric hypoxia is, on the one hand, a compensatory increase in the production of red blood cells<sup>9-11</sup> to ensure an adequate supply of oxygen to the tissues and, on the other hand, a decrease of plasma volume,<sup>9-11</sup> an adaptation that lasts throughout the stay at high altitudes. The production of red blood cells is stimulated by the increase of erythropoietin in plasma, which stimulates the proliferation and development of stem cells in the bone marrow and their transformation into red line cells. The increase of the erythrocytic mass produces an increase of blood viscosity, which can provoke a decrease in blood circulation and the supply of oxygen to the tissues.<sup>8</sup>

The increase in hemoglobin concentration in relation to altitude was studied in the 1940s by Hurtado. His work with adult men<sup>5</sup> led Dallman et al.<sup>12</sup> to suggest an adjustment of the reference cut-off values established at sea level<sup>13</sup> to include a 4% increase in the concentration of hemoglobin per 1000 m elevation, being a linear relation. These reference values are currently used for the detection of anemia in high-altitude countries.

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Hurtado's work demonstrates, however, that the curve of the increased concentration of hemoglobin with altitude is not linear, but exponential. This is a result of the existing curvilinear relation between oxygen bound to hemoglobin and the change in partial oxygen pressure with altitude.<sup>8</sup> To maintain a constant quantity of oxygen, the concentration of hemoglobin would increase exponentially with the linear increase in altitude. This is confirmed by two recent studies. In 1979, Arnaud<sup>14</sup> studied hematopoiesis at different altitudes and demonstrated that the evolution of the concentration of hemoglobin in relation to altitude is different above and below 3000 m. Freire et al. showed that the curve of the concentration of hemoglobin in children 6 months to 5 years old without iron deficiency and living at different altitudes is exponential and parallel to the curve found by Hurtado for altitudes lower than 3500 m, with a sharper inflection above 3000 m.<sup>15,16</sup> The linear correction of the concentration of hemoglobin with altitude seems to lack biologic support, and is therefore inappropriate for the diagnosis of anemia at high altitudes.

The inadequacy of the corrections recommended by Dallman et al.<sup>12</sup> have been confirmed by a series of studies conducted in the Andean region. The diagnosis of anemia in adult men residing in La Paz (3600 m) establishes a cut-off value of 158 g/L,<sup>17</sup> higher than Dallman's corrected cut-off value according to altitude (149 g/L). Other studies conducted on children in Ecuador and Bolivia demonstrate that the cut-off values for the diagnosis of anemia are higher than those recommended.<sup>15,18-20</sup> A report by the U.S. Centers for Disease Control and Prevention (CDC),<sup>21</sup> based on analysis of data from the CDC Pediatric and Pregnancy Nutrition Surveillance System and concerning North American populations of high-altitude states, proposes corrections for altitudes similar to those resulting from the studies of the Andean regions. The diagnosis of anemia based on a positive response to an iron supplement in children and women of childbearing age indicates that the use of recommended cut-off values<sup>12</sup> introduces an underestimation of the real prevalence of anemia.<sup>15,18-20,22</sup>

Around 20-30 million people worldwide live at altitudes higher than 3000 m, defined as high altitude,<sup>23</sup> especially in the high plains of Ethiopia and the Tibetan plateau of the Himalayas, where the adaptation of life to high altitudes could occur without an increase in the concentration of hemoglobin,<sup>24</sup> and above all in the Andean high plateau. Approximately 17 million people live at high altitudes in the Andean region of Latin America,<sup>25</sup> and 38% of them are Bolivians.<sup>26</sup>

In Bolivia, data on the prevalence of anemia are poor. One report by UNICEF states that the prevalence is approximately 48-50% in children less than 15 years of age residing in Cochabamba (2400 m).<sup>27</sup> The same report indicates that anemia is very uncommon in the Bolivian altiplano, which seems unlikely in view of two recent studies

that found a prevalence of anemia oscillating between 67.2% and 14.6% in children between 6 months and 9 years of age,<sup>20</sup> and around 56.5% in pregnant women.<sup>22</sup> This discrepancy might be due to the utilization of different cut-off values for the definition of anemia.

The preceding information lends urgency to the need to define cut-off values for the concentration of hemoglobin used to define anemia in populations residing at high altitudes. The objective of our study was to establish, through an iron-folate supplementation trial, the cut-off values that reveal the existence of anemia in women of childbearing age residing at high altitudes, as well as to estimate the prevalence of anemia in the above-mentioned populations.

## Methodology

### Region of Study

The study took place in two rural populations of the Bolivian altiplano in the region of Potosí: Atocha (3600 m) and Santa Bárbara (4800 m). It lasted from February to September, during the cold and dry winter season.

### Subjects

The criteria for inclusion were the following: 15-40-year-old women, nonpregnant, well-nourished, not suffering from chronic illness and/or acute infection, residing in the study region at least the 2 previous years, and no plans to leave the study region during the following 6 months.

The sample size was estimated to achieve precision in the mean hemoglobin concentration of 2.5 g/L with a probability of error of 0.05. The standard deviation of the concentration of hemoglobin (10 g/L) was estimated from studies conducted on similar populations. The required sample size was 62 women per group. The number of anticipated withdrawals for reasons of rejection or migration was estimated at 23 per group, which is why it was considered necessary to include 85 women in each group. A census of women of childbearing age was done in both locations. The women included in the study were randomly selected from the eligible women in each population.

Sample size calculation for the control group indicated that at least 30 women were required to discern a difference in hemoglobin between supplemented and control groups equal or greater than 10g/L with a type I error of 0.05 and a power of 0.90, and a one-tailed *t* test.

### Study Design

Intervention included daily supplementation (6 days/week) for 3 months with tablets containing 3 mg elemental iron and 20 µg folic acid/kg body weight/day. The tablets were administered with cooled boiled water 2 hours after the last meal and at least 1 hour before the subsequent meal. Fourteen local assistants were responsible for the administra-

**Table 1.** Hematologic and Anthropometric Values Before and After Supplementation, Santa Bárbara (4800 m)

Variable	T <sub>0</sub>		T <sub>3</sub>		p Between T <sub>0</sub> and T <sub>3</sub> *	
	Iron-Folate	Control	Iron-Folate	Control	Iron-Folate	Control
Age (years)	27.8 + 7.2	28.7 + 6.0	—	—	—	—
Hemoglobin (g/L)	177.2 + 22.3	181.1 + 23.0	191.7 + 18.8	184.9 + 22.3	0.0000	NS
Hematocrit (%)	55.0 + 6.6	56.3 + 6.5	58.1 + 6.3	56.1 + 5.9	0.0012	NS
Red blood cells (10 <sup>6</sup> /mm <sup>3</sup> )	5.159 + 0.613	5.229 + 0.653	5.380 + 0.664	5.399 + 0.657	0.0049	NS
EPP (μg/g Hb)	2.59 + 1.50	1.97 + 0.73	2.06 + 0.89	2.19 + 0.77	0.0002	NS
Weight (kg)	52.4 + 9.0	52.2 + 8.5	53.7 + 9.8	52.6 + 9.0	NS	NS
Height (cm)	149.5 + 6.9	148.2 + 6.4	149.5 + 6.9	148.7 + 6.5	NS	NS
BMI (kg/m <sup>2</sup> )	23.4 + 3.3	23.8 + 3.9	24.1 + 5.3	23.8 + 3.8	NS	NS

\*Paired *t* test.

tion of the tablets. The assistants' work was strictly controlled by a general supervisor.

### Blood Analysis

Hematologic values were determined from total blood samples obtained from the finger tip before (T<sub>0</sub>) and after (T<sub>3</sub>) supplementation. Concentration of hemoglobin, hematocrit, and numbers of red blood cells and leukocytes were determined by means of a cell counter (Coulter Counter M530 from Coulter Electronics Ltd., England) with blood standards M530. A hematofluorometer (AVIV Biomedical Inc., Lakewood, New Jersey) was used to determine erythrocyte protoporphyrin (EPP).

### Evaluation of Nutritional Status

A general clinical exam was done the day of the blood analysis. Height and weight were anthropometric measurements used to assess nutritional status. Weight was measured with a platform scale (TEFAL SC 3301) with a precision to 200 g and height with a precision to 1 mm. Each woman's body mass index (BMI, weight in kilograms divided by height in meters squared) was then determined.

All of the women who participated in the study did so in a free and informed manner. The study's protocol was presented to and approved by the Scientific Committee of the Bolivian Institute of High Altitude Biology (IBBA).

## Results

### Effect of Supplementation

In Santa Bárbara (4800 m), the hematologic and nutritional status of the iron-folate and control groups, constituted by random selection (see Table 1), were identical (*t* test) at T<sub>0</sub>. The hematologic values before and after supplementation were compared in each group (paired *t* test). The iron-folate supplementation produced a significant increase in the concentration of hemoglobin, hematocrit, and the number of red blood cells and a decrease in the concentration of EPP. In the control group, however, no significant difference was observed. The increase in hemoglobin concentration was significantly higher in the supplemented group, which also showed a greater decrease in EPP values, proving the positive effect of the supplement.

In Atocha (3600 m), comparison of hematologic values before and after supplementation reveals a positive but statistically insignificant effect (Table 2).

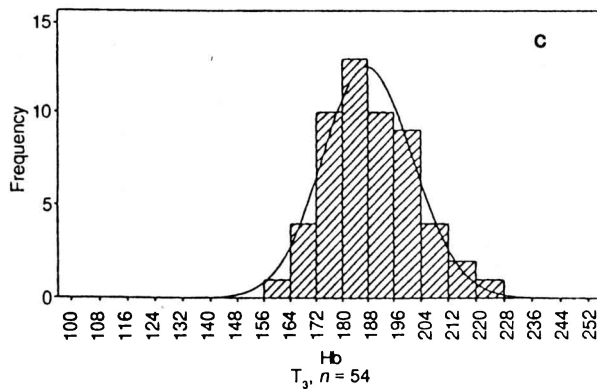
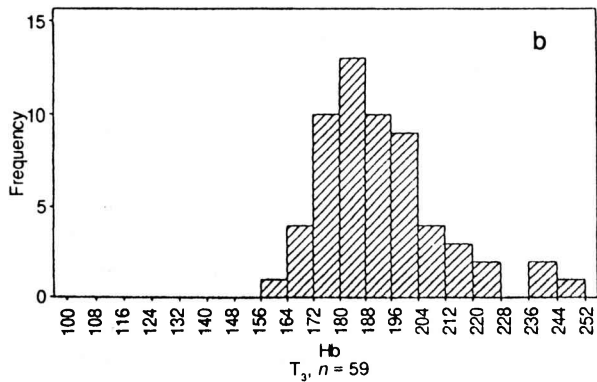
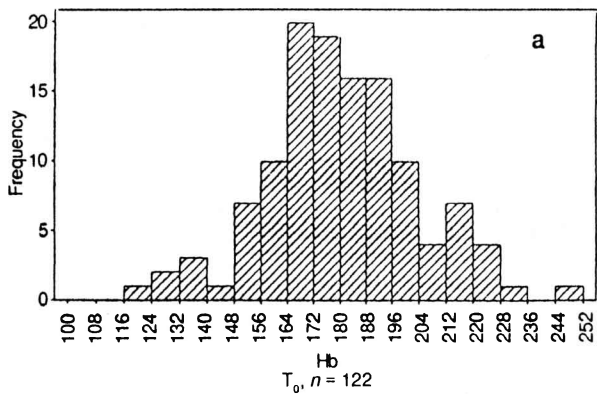
### Analysis of Hemoglobin Distributions

In Santa Bárbara, the population could be divided into three groups according to hemoglobin concentration: anemic, normal, and polycythemic. Supplementation with iron and folate yielded a population of women without deficiency of these nutrients and thus, in principle, not ane-

**Table 2.** Hematologic and Anthropometric Values Before and After Supplementation, Atocha (3600 m)

Variable	T <sub>0</sub>	T <sub>3</sub>	p Between T <sub>0</sub> and T <sub>3</sub> *
	Age (years)	29.2 + 7.2	—
Hemoglobin (g/L)	164.0 + 17.3	166.3 + 12.2	NS
Hematocrit (%)	49.7 + 5.0	50.4 + 3.9	NS
Red blood cells (10 <sup>6</sup> /mm <sup>3</sup> )	4.675 + 0.360	4.714 + 0.51	NS
EPP (μg/g Hb)	2.25 + 1.26	2.11 + 0.65	NS
Weight (kg)	51.8 + 8.6	52.8 + 7.9	NS
Height (cm)	150.8 + 4.6	151.1 + 4.4	NS
BMI (kg/cm <sup>2</sup> )	23.0 + 3.7	23.0 + 3.0	NS

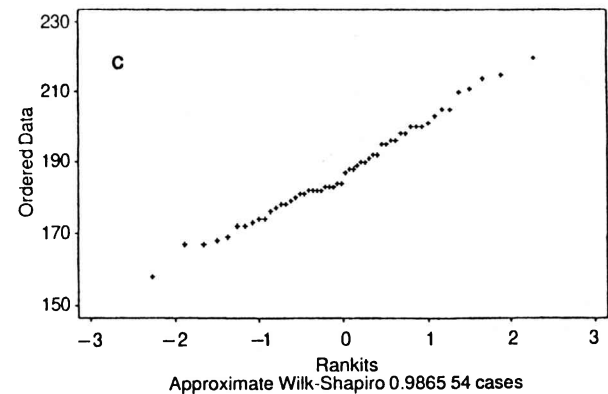
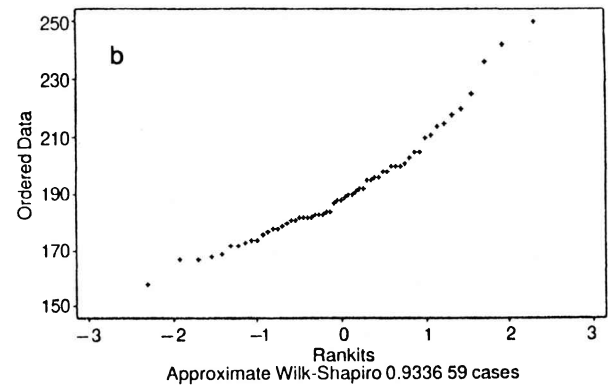
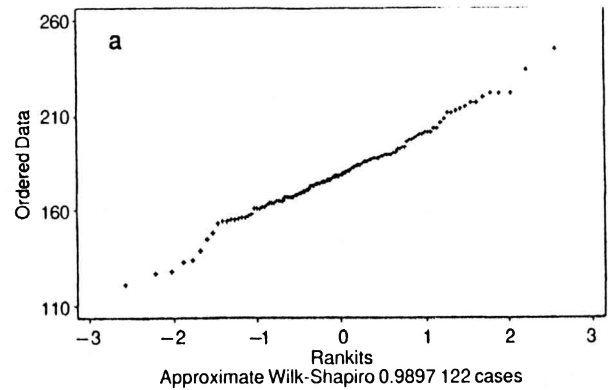
\*Paired *t* test.



**Figure 1.** Histograms of the distribution of the hemoglobin concentration (g/L) in Santa Bárbara: (a) before supplementation, (b) after supplementation, and (c) after supplementation and exclusion of polycythemic women.

mic. Infection was controlled for by eliminating from the analysis all women who presented with a concentration of leukocytes higher than  $10,000/\text{mm}^3$  blood. Figures 1 and 2 show a deviation with respect to normal only for high hemoglobin values. The distribution of the concentration of hemoglobin obtained by the exclusion of women thought to have polycythemia ( $\text{Hb} > 220 \text{ g/L}$ ) is normal (Wilk-Shapiro test = 0.99).

In Atocha, the distribution of hemoglobin concentration before supplementation is skewed to the left, toward the lowest hemoglobin values, which are visible on Figures 3 and 4. The high hemoglobin values do not deviate with respect to the straight line, which indicates the absence of polycythemia in this population. After supple-



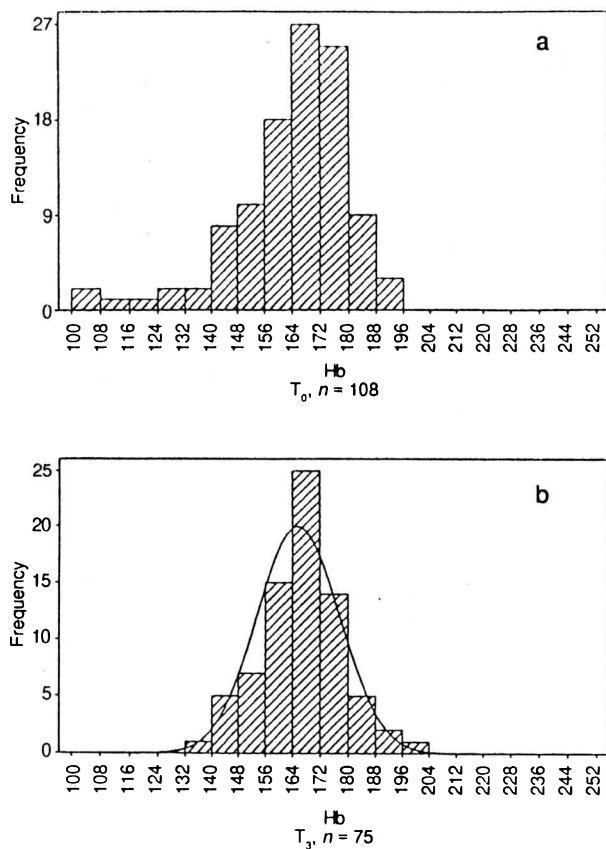
**Figure 2.** Distribution of the hemoglobin concentration (g/L) in Santa Bárbara: (a) before supplementation, (b) after supplementation, and (c) after supplementation and exclusion of polycythemic women.

mentation and exclusion of women who presented with a concentration of leukocytes higher than  $10,000/\text{mm}^3$  blood, the distribution was normal (Wilk-Shapiro test = 0.98).

#### **Parameters of the Hemoglobin Distribution and Cut-off Values**

The 2.5th percentile of the distribution of hemoglobin of the study population allows for the estimation of the cut-off value of hemoglobin for the definition of anemia. The  $\text{Hb}_0$  value is therefore determined as  $P(\text{Hb} < \text{Hb}_0) = 2.5\text{th}$  percentile of the distribution of hemoglobin in the population. With the same procedure, the cut-off value for polycythemia is estimated on the basis of the 97.5th percentile.

In Santa Bárbara the cut-off for the definition of ane-



**Figure 3.** Histograms of the distribution of the hemoglobin concentration (g/L) in Atocha (3600 m): (a) before supplementation and (b) after supplementation.

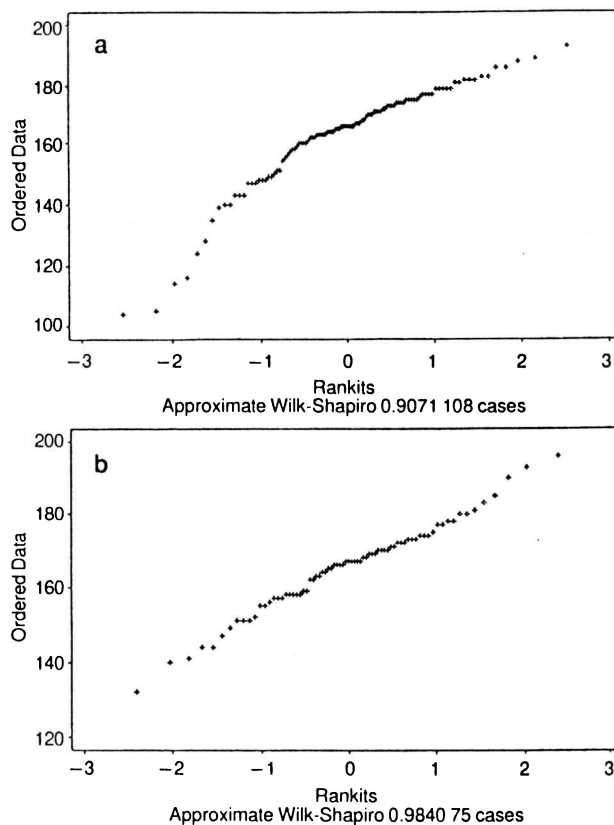
mia is estimated at 160.9 g/L and in Atocha at 142.0 g/L. The mean values of the distribution were 187.7 and 165.6 g/L, respectively, and the cut-off values above which one can suspect polycythemia are 214.6 and 189.1 g/L, respectively.

The estimation of the parameters of the distribution can also be obtained by a graphic method that represents the cumulative probabilities of hemoglobin concentrations.<sup>17,25,28,29</sup> The cut-off values for anemia estimated by this method are, overall, inferior (i.e., 135 g/L at 4800 m and 137.8 g/L at 3600 m), with the mean values of the distribution 179.5 and 166.3 g/L and the cut-off values of polycythemia 225.0 and 194.5 g/L, respectively, for the two altitudes.

#### Effectiveness of the Cut-off Values

The effectiveness of a cut-off value depends on its capacity to identify individuals who are anemic and those who are not. The sensitivity is the capacity of the cut-off value to diagnose an anemic person as anemic; the specificity is its capacity to identify a nonanemic person as nonanemic. Individuals were defined as truly anemic, or responders, when their concentration of hemoglobin increased at least 10 g/L pre- and postsupplementation.<sup>18,30,31</sup>

The sensitivity of the cut-off value of anemia based



**Figure 4.** Distribution of the hemoglobin concentration (g/L) in Atocha: (a) before supplementation and (b) after supplementation.

on the 2.5th percentile of the distribution is less than 30% for both altitudes. A test of low sensitivity does not allow the identification of a considerable proportion of anemic individuals. Tables 3 and 4 present different cut-off values chosen to calculate the sensitivity and the specificity of the hemoglobin, allowing for the construction of the receiver operating characteristic (ROC) curves, sensitivity versus 1-specificity,<sup>32</sup> and the estimation of the optimum cut-off value (Figure 5). The farther the ROC curve is from the straight line of probability, which links the two opposite angles, the higher the efficacy of the test. The optimum cut-off value is obtained from the point on the ROC curve nearest the upper left corner.<sup>33</sup> The cut-off values obtained from the ROC curves are 170.0 g/L for 4800 m and 162.0 g/L for 3600 m.

#### Prevalence of Anemia

The prevalence of anemia, measured as the percentage of individuals responding to supplementation, is 51.7% in Santa Bárbara and 26.5% in Atocha. The prevalence was also calculated for Santa Bárbara according to Garby et al.'s method,<sup>34</sup> which takes into account the regression toward the mean when there are two measures for the same variable. This method compares, for each level of hemoglobin before supplementation, the hemoglobin response

**Table 3.** Performance of Hemoglobin Cut-off Values and Prevalence of Anemia, Santa Bárbara (4800 m)

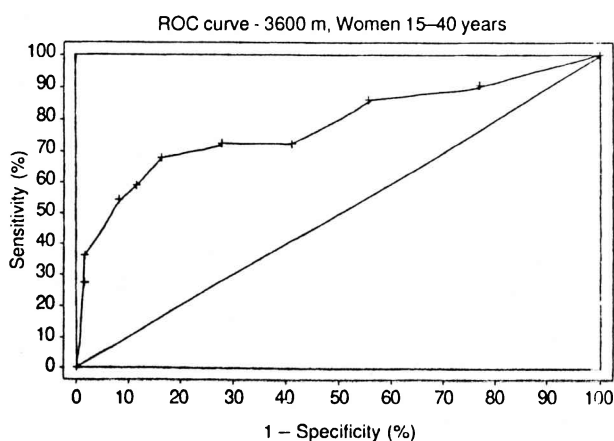
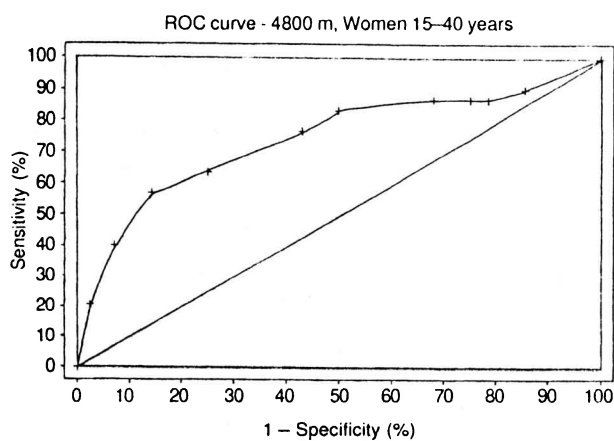
Cut-off Values (g/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Measured Prevalence (%)
160.9	26.7	100.0	81.8	60.9	14.9
165	40.0	92.9	85.7	59.1	23.4
170	56.7	85.7	81.0	64.9	35.1
175	63.3	75.0	73.1	65.6	43.6
180	76.7	57.1	65.7	69.6	55.3
185	83.3	50.0	64.1	73.7	61.7
190	86.7	32.1	57.8	69.3	70.2

Note: PPV and NPV = positive and negative predictive values. True prevalence = positive response to supplementation: 51.7%; Garby et al.'s method (reference 34): 51.7%.

**Table 4.** Performance of Hemoglobin Cut-off Values and Prevalence of Anemia, Atocha (3600 m)

Cut-off Values (g/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Measured Prevalence (%)
142.0	27.3	98.4	85.7	79.0	8.5
147	36.4	98.4	88.9	81.1	11.3
152	54.6	91.8	70.6	84.9	21.7
157	59.1	88.5	65.0	85.7	24.5
162	68.2	83.6	60.0	87.9	33.0
165	72.7	72.1	48.5	88.0	44.3
167	72.7	59.0	39.0	85.7	52.8

Note: PPV and NPV = positive and negative predictive values. True prevalence = positive response to supplementation: 26.5%.

**Figure 5.** Receiver operating characteristic (ROC) curves of hemoglobin.

to supplementation with iron-folate with those same values for the control group. The linear regression for hemoglobin-after/hemoglobin-before is based on the control group. The subjects from the iron-folate group are compared with the straight line of the obtained regression; the number of subjects under this straight line represents half of those who were nonresponders.

The linear regression obtained from the control group of Santa Bárbara is:

$$\text{Hemoglobin after} = 123.752 + (0.31044 \times \text{Hemoglobin before}).$$

The number of individuals in the iron-folate-supplemented group who are under the straight regression line is 14, which means that 28 were nonresponders. The prevalence of anemia using this method is 51.7%, which is identical to the prevalence estimated by the response to supplementation of at least 10 g/L.

## Discussion

The prevalence of anemia in a population can be estimated by different methods. The criterion most appropriate for the diagnosis of nutritional anemia at the individual level is an increase in hemoglobin concentration greater than or equal to 10 g/L, as a response to supplementation with iron-folate.<sup>18,30,31</sup> The 3-month supplementation of our study met the iron and folate requirements of the women of the study, as confirmed by the improvement in the hematological parameters.

The prevalence of anemia, determined by a response

to supplementation of at least 10 g/L, is 51.7% in Santa Bárbara and 26.5% in Atocha. Nutritional anemia is established, therefore, as a public health problem in women of childbearing age in the Bolivian altiplano. The estimated prevalence according to Garby et al.'s method,<sup>34</sup> which controls for the effect of regression of the concentration of hemoglobin toward the mean of two measures, is 51.7%, identical to the estimated prevalence according to the positive response to the supplementation. This demonstrates that both methods show comparable estimated numbers of responders.

The prevalence of anemia may also be estimated by the analysis of the mixed distribution of hemoglobin that involved the plotting of cumulative frequency distributions of hemoglobin on probability paper.<sup>35-36</sup> If the distribution of the hemoglobin is perfectly normal (population of healthy and well-nourished subjects) the cumulative probability of the hemoglobin is a straight line.<sup>17,25,28,29</sup> The presence of anemic and polycythemic individuals in the population of Santa Bárbara induces a deviation in the straight line at its two extremes. This clearly shows that in this location there are three populations: anemic, normal, and polycythemic. In Atocha, the diagram of cumulative probability demonstrates a curvilinear deviation only in the lower extreme, which seems to indicate that polycythemia does not affect the women living at an altitude of 3600 m, as confirmed by the study by Moreno-Black et al.<sup>29</sup> on a sample of 152 women living at the same altitude.

The prevalence of anemia estimated by analysis of the mixed distribution (estimated by the difference between the inferior curvilinear portion and the extrapolated linear portion) is 2.8% in Santa Bárbara and 6% in Atocha, values far below those estimated according to the response to supplementation and similar to the prevalence of 3% found in the study by Moreno-Black et al.<sup>29</sup> A study conducted in Nepal<sup>24</sup> found that the estimated prevalence according to the method of mixed-distribution analysis is approximately four times lower than the estimation based on the adjusted cut-off value for altitude proposed by the CDC,<sup>21</sup> which led the authors to issue the hypothesis that Tibetans have a different hemoglobin adaptation response. Our study is the only of those mentioned here that includes a supplementation trial with iron and folate. This supplementation allowed us to demonstrate that the use of the method of mixed-distribution analysis for hemoglobin considerably underestimates the prevalence of anemia in the study populations.

The conventional focus in estimating the prevalence of anemia in a population is based on identifying the proportion of individuals whose concentration of hemoglobin is lower than a cut-off value defined in relation to sex, age, and the physiologic condition of the individual.<sup>13</sup> These cut-off values are, after correction at the rate of 4% for each 1000 m of altitude,<sup>12</sup> 143.0 g/L for 4800 m and 137.3 g/

L for 3600 m. The prevalence is considerably underestimated: 14.7% in Santa Bárbara and 5.7% in Atocha. The sensitivity of these cut-off values is only 13.3% and 22.7%, respectively, its specificity being 100%. Our study demonstrates the lack of effectiveness of the WHO cut-off values when corrected for altitude, which have been presented as evidence for various studies.<sup>15,19,31</sup> The Ecuador study indicates that the sensitivity of the WHO-proposed cut-off values for altitude is 58% for a population residing at sea level and 0% for a similar population living at 2800 m.<sup>31</sup> The linear correction of 4% for each 1000 m is, therefore, obsolete and new cut-off values need to be defined.

The definition of a cut-off value for hemoglobin that permits the diagnosis of anemia requires a reference population consisting only of healthy individuals<sup>7</sup> free of all nutritional deficiency that could influence the concentration of hemoglobin. The definition of norms and the determination of the distribution curve of the frequencies of normal hemoglobin concentrations are possible only by excluding individuals with nutrient deficiencies at specific levels or by administering antianemic supplements.<sup>6,30,32,37</sup> Supplementation with hematopoietic nutrients is recommended by several authors.<sup>15,18,19</sup> When it is not possible to determine with certainty the etiology of nutritional anemia, all experiments with supplements ought to simultaneously include iron and folic acid.<sup>38</sup> The supplementation with iron and folate in our study allowed for the elimination of nutritional anemia. Once the women with suspected infections were excluded, the distribution of hemoglobin obtained by the supplementation was normal and was then used to define the cut-off values.

Although the defined cut-off values in our study based on the 2.5th percentile of the distribution of hemoglobin presented a sensitivity much greater than the values of WHO, they are still low. To increase sensitivity implies a loss of specificity. The use of the ROC curves<sup>32</sup> allowed us to define an optimum cut-off value of 162.0 g/L for an altitude of 3600 m, with a sensitivity and specificity of 68.2% and 83.6%, respectively, and an optimum cut-off value of 170.0 g/L for an altitude of 4800 m, with a sensitivity of 56.7% and a specificity of 85.7%.

The choice of a cut-off value depends on, in addition to its effectiveness, the desired objective, the chosen strategies, and the available means (detection of all individuals at risk of anemia, dealing only with anemic individuals, estimating the prevalence of anemia closest to reality, etc.). The sensitivity and specificity of the chosen cut-off value permit the estimation of the real prevalence of anemia<sup>15</sup> based on the formula:

$$PR = (\text{measured prevalence} + \text{specificity} - 1) / (\text{sensitivity} + \text{specificity} - 1).$$

The use of this formula is the equivalent of eliminating false positives and adding the false negatives to the measured prevalence, which corresponds to the calculation



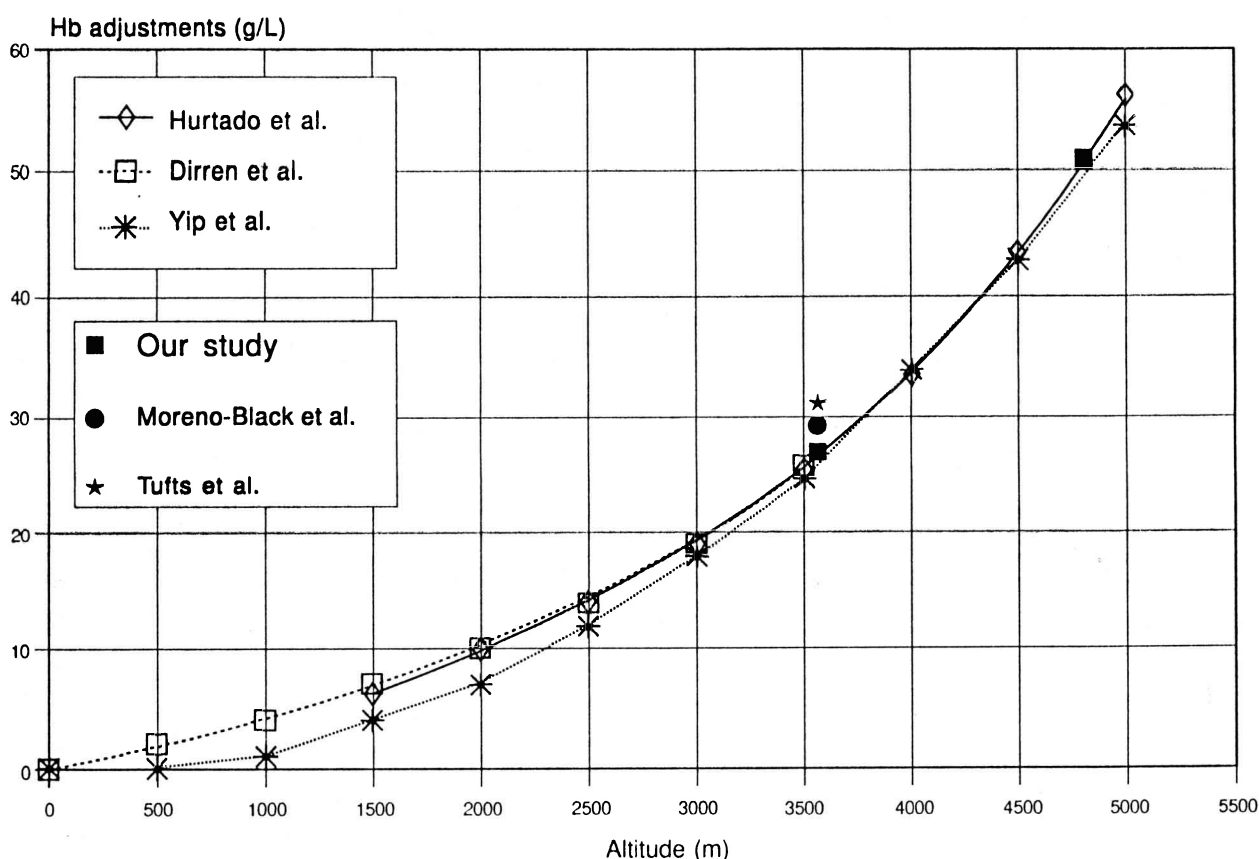


Figure 6. Hemoglobin-altitude curve.

method proposed by Mora<sup>39</sup> to determine the standardized prevalence of malnutrition. This method is not based on a cut-off value, but on the comparison of two populations, the study and the reference populations, assuming that these populations are approximately normal and that the distance between the two is expressed as a z score. This method is, therefore, independent of any cut-off value and can be used only to estimate prevalence. Work analogous to that of Mora could be carried out for anemia if the availability of reference populations comparable for age and sex can be corrected for with respect to altitude.

The relation between the concentration of hemoglobin and altitude was studied in the 1940s by Hurtado et al.<sup>5</sup> They demonstrated that the curve of the increase in the concentration of hemoglobin in relation to altitude is exponential, which was confirmed by a later study by Dirren et al.<sup>16</sup> in Ecuadorian children. The curve of the increase in hemoglobin in Ecuadorian children is parallel to the curve of Hurtado et al. for altitudes lower than 3000 m, but its extrapolation for higher altitudes would present a sharp increase. A 1989 report of the CDC Pediatric and Pregnancy Nutrition Surveillance System<sup>21</sup> of the North American population residing in high altitudes showed that the relation between the increase in hemoglobin and altitude is curvilinear:  $Hb = -0.105a + 0.236a^2$ , with  $a =$  an elevation of 1000 m.<sup>40</sup> The adjustments obtained were slightly lower

than those proposed by Dirren et al., especially those that refer to lower altitudes.

These two studies have only considered individuals living in moderate altitudes: 0–3400 m in Dirren et al.'s study and 0–2500 m in the CDC study. With the exception of the study by Hurtado et al., few studies have been conducted at altitudes higher than 3000 m. Our study presents the adjustments based on the mean values of the distributions of hemoglobin of subjects without anemia and polycythemia. The estimated adjustments based on our distributions are similar but slightly lower than those calculated in the study by Hurtado et al. (30.6 versus 33.5 g/L for 3600 m and 52.7 versus 57.5 g/L for 4800 m, respectively).

Two other studies have examined the distribution of hemoglobin in apparently healthy women<sup>29</sup> and men<sup>17</sup> residing at an altitude of 3600 m. The adjustments obtained from the mean value of hemoglobin defined by mixed distribution analysis are 32.5 g/L for women and 35.0 g/L for men. The graphic representation of the adjustments of hemoglobin obtained or estimated at different and complementary altitudes (Figure 6) invalidates the linear correction of Dallman et al.<sup>12</sup> and shows that these studies meet a consensus regarding the exponential hemoglobin-altitude relationship of Hurtado et al. that can be used for the adjustment to different altitudes of the reference populations defined at sea level.



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