Adrenopause or decline of serum adrenal androgens with age in women living at sea level or at high altitude

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Abstract

The present study aimed to determine adrenopause or reduction of serum adrenal androgens with age at high altitude and at sea level. It was a cross-sectional study performed in 210 women resident at high altitude (4340 m) and 123 women living in Lima (150 m), aged 20–70 years. Fasting early morning blood samples were obtained. Serum dehydroepiandrosterone (DHEA), DHEA sulphate (DHEAS), androstenedione, testosterone and estradiol were measured by radioimmunoassay. Serum testosterone concentrations were greater in women living at high altitude than in those resident at sea level. Serum concentrations of DHEA, DHEAS and androstenedione were lower in women living at high altitude than in those living at sea level. The DHEAS/DHEA ratio was significantly greater, and the androstenedione/testosterone ratio was lower in samples from women living at high altitude. Among women older than 50 years of age, a greater decline in serum concentrations of DHEA was observed in those living at high altitude than in those living at sea level. Among women 60–70 years of age, serum concentrations of DHEA at high altitude were 46·9% of those in women of the same age living at sea level. Decay of DHEAS at sea level and at high altitude occurred from the age of 40 years. The decline was faster at high altitude than at sea level, and in women aged 60–70 years serum values of DHEAS at high altitude were 56% of those at sea level. In the same age group, serum concentrations of androstenedione among those native to high altitudes were 27·34% of the value at sea level. At sea level, serum testosterone concentrations did not change with age from 20 to 70 years. In women aged 20–39 years and 50–59 years, serum testosterone concentrations were greater at high altitude than at sea level (P<0·05). In those aged 60–70 years, the concentrations were similar in those living at sea level and at high altitude. At sea level and at high altitude, the serum testosterone/estradiol ratio increased with age (P<0·0034 and P<0·0001 respectively). This ratio increased at an earlier age among those living at high altitude (40–49 years) than among those living at sea level (50–59 years). Multivariate analysis showed that altitude (P<0·0001) and greater chronological age (P<0·0001) were associated with lower serum DHEAS concentrations. DHEAS was related to chronological age (P<0·0001). Low serum androstenedione concentrations were related to living at high altitude at birth and greater chronological age (P<0·0001). In conclusion, adrenopause is attained earlier and is of greater magnitude at high altitude than at sea level.


Introduction

Human aging involves a series of chronological processes including shortening of telomeres of the chromosomes, mitochondrial aging, accumulation of mutations, and expression of genes of aging, among others (Bowles 1998). In women, aging is accompanied by a decline in circulating concentrations of components of the growth hormone–insulin–like growth factor I axis (Corpas et al. 1993), the adrenal sex steroid precursors – dehydroepiandrosterone (DHEA) and its sulphated ester (DHEAS) – and ovarian sex hormones (Birkenhager-Gillesse et al. 1994, Lamberts et al. 1997, Semezuk 1998). These processes define somatopause, adrenopause and menopause respectively. Somatopause and adrenopause are associated with general aging (Ravaglia et al. 1996, Morales et al. 1998), whereas menopause is the expression of reproductive aging (Soules et al. 1998, Te Velde et al. 1998a, b). There is some evidence that both general aging and reproductive aging are related (Dorland et al. 1998, Kirkwood 1998). Furthermore, there is a strong association between age at natural menopause and age at death (Snowdon et al. 1989, Snowdon 1990, WHO 1996).

DHEA and its sulphated metabolite DHEAS are the main products of the zona reticularis of the human adrenal glands (Birkenhager-Gillesse et al. 1994). DHEA is...
quantitatively the most important steroid in the human body (Vermeulen 1997). It has been suggested that DHEA may intervene in processes of maturation (Góñez et al. 1993) and aging (Vermeulen 1997, Morales et al. 1998).

Serum concentrations of DHEA decline significantly, starting from the age of 30 years, when the maximal value is attained (Labrie et al. 1997). Treatment with DHEA for 3 (Morales et al. 1994) or 6 months (Morales et al. 1998) resulted in improvement of physical and psychological well-being in aged men and women.

Gonzales & Villena (1997) and León-Velarde et al. (1997) have demonstrated that median age at menopause occurs earlier at high altitudes in the Central Andes than at sea level in Peru. It is known that women of a younger age at menopause also have a lower life expectancy (WHO 1996). In Cerro de Pasco at 4340 m, located in the central Andes of Peru, life expectancy is shorter than at sea level (León-Velarde & Arregui 1994).

According to these data, general aging may be attained early in populations living at high altitudes. If aging is occurring early at high altitude, and if a relationship between serum adrenal androgen concentrations and age is accepted, it is possible that the decline in serum adrenal androgen concentrations may occur earlier in women living at high altitude than in those at sea level.

The present study was designed to determine serum concentrations of adrenal androgens (DHEA, DHEAS and androstenedione), testosterone and estradiol in women of different ages living at sea level and at high altitude.

Materials and Methods

Study design

This was a cross-sectional study comparing women of different ages resident at sea level and at high altitude. During sample analysis, serum collected from women living at both sea level and high altitude were included in the same assay run, to reduce between-assay variation.

Study participants

The study was performed in 210 women who were native to high altitude, residing at Cerro de Pasco, Peru (4340 m) and aged 20–70 years, who had lived in the place of study for at least 10 years without significant migrations to or from different altitudes. As a control group, we studied 123 women aged 20–70 years, living in Lima at 150 m above sea level. The women were grouped according to age: 20–29 years, 30–39 years, 40–49 years, 50–59 years and >59 years. We chose to study about 20 women for each age group at sea level and double the number at Cerro de Pasco.

Women from Lima were of the same ethnic group as those at high altitude.

Data were obtained through a structured interview or by written questionnaires. They included chronological age, place of birth, altitude of birthplace, age at first and last menses, and total number of pregnancies. Smoking was observed in fewer than 1% of the women included in the study.

Menopause was defined as cessation of menses at least 12 months before the interview. Postmenopausal women receiving hormone replacement therapy were excluded from the study. None of those included in the study had been taking any medication during the last 3 months before the study.

Height and weight were measured in each woman. Body mass index (BMI) was calculated according to the equation weight (kg)/height (m)².

A fasting venous blood sample was obtained from each woman between 08:00 and 10:00 h, to avoid circadian variations. In premenopausal women, the part of the cycle chosen for blood sampling was not different between those living at sea level and at high altitude (mean ± s.d. day of cycle: 13·80 ± 9·60 and 16·07 ± 10·15, at high altitude and sea level respectively; NS).

Measurements of serum estradiol and testosterone concentrations

Serum estradiol and testosterone concentrations were measured by radioimmunoassay using commercially available kits (Diagnostic Products Co., Los Angeles, CA, USA): catalogue number TKE25 for estradiol and TKTT5 for testosterone. The within-assay variations were 6·4% for estradiol and 5·5% for testosterone. Sensitivity of the testosterone assay was 4·0 pg/ml and that for the estradiol assay was 1·5 pg/ml.

Measurements of DHEA, DHEAS and androstenedione concentrations

The serum adrenal androgen concentrations were measured by RIA using commercial kits (Diagnostic Products Co.): catalogue number TKDS5 for DHEAS, TKDH2 for DHEA and TKAN2 for androstenedione. DHEA was assayed after anhydrous ethyl ether extraction. For measurement of DHEAS and androstenedione, no extraction procedure was required. The within-assay variations were 4% for DHEAS, 7% for DHEA and 5% for androstenedione.

The DHEA/androstenedione ratio was used as marker of 3β-hydroxysteroid dehydrogenase activity. DHEAS/DHEA, androstenedione/testosterone, and testosterone/estradiol ratios were also calculated, as markers of the metabolism of DHEAS, androstenedione and testosterone respectively.

Statistical analysis

Data were analysed using the STATA (version 4·0) program (Stata Corporation, College Station, TX, USA).
Table 1 Selected physiological variables at sea level and at high altitude. Data are mean ± S.D.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sea level</th>
<th>High altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43·62 ± 12·31</td>
<td>42·84 ± 10·95</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>13·30 ± 1·63</td>
<td>14·29 ± 1·56**</td>
</tr>
<tr>
<td>Age at menopause (years)</td>
<td>47·00 ± 3·28</td>
<td>45·31 ± 3·13*</td>
</tr>
<tr>
<td>Post menopausal years</td>
<td>10·35 ± 5·09</td>
<td>8·28 ± 6·80</td>
</tr>
<tr>
<td>Pregnancies (n)</td>
<td>4·68 ± 3·26</td>
<td>6·22 ± 3·72**</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26·34 ± 4·00</td>
<td>25·88 ± 4·11</td>
</tr>
<tr>
<td>Pulse oxygen saturation (%)</td>
<td>98·39 ± 1·83</td>
<td>87·73 ± 3·83**</td>
</tr>
<tr>
<td>Serum testosterone (pg/ml)</td>
<td>62·61 ± 63·09</td>
<td>95·02 ± 106·86**</td>
</tr>
<tr>
<td>Serum estradiol (pg/ml)</td>
<td>63·21 ± 69·99</td>
<td>79·45 ± 99·23</td>
</tr>
<tr>
<td>DHEA (ng/ml)</td>
<td>1·99 ± 1·27</td>
<td>1·22 ± 1·40**</td>
</tr>
<tr>
<td>DHEAS (µg/ml)</td>
<td>149·7 ± 82·7</td>
<td>90·9 ± 63·6**</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>1·66 ± 0·62</td>
<td>1·00 ± 0·86**</td>
</tr>
<tr>
<td>DHEAS/DHEA ratio</td>
<td>87·29 ± 47·02</td>
<td>149·12 ± 145·14**</td>
</tr>
<tr>
<td>DHEA/androstenedione ratio</td>
<td>0·99 ± 0·44</td>
<td>1·41 ± 1·82</td>
</tr>
<tr>
<td>Androstenedione/testosterone ratio</td>
<td>7·10 ± 8·52</td>
<td>1·97 ± 2·86***</td>
</tr>
</tbody>
</table>

DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate. *P<0·05; **P<0·01; ***P<0·0001 with respect to values at sea level (Student’s t-test).

For quantitative data, homogeneity of variances was assessed by the Bartlett test. If variances were homogeneous, differences between groups were assessed by one-way analysis of variance. Differences between pair of means were assessed by the Scheffé test. When variances were not homogeneous, the Wilcoxon range test was used.

Single and multiple linear regression analyses were performed to assess the independent effect of age, hormone concentrations and BMI on serum concentrations of adrenal androgens.

A value of P<0·05 was considered to be statistically significant.

Results

Chronological age, postmenopausal years, BMI and serum concentrations of estradiol were similar in the samples collected from women living at sea level and those living at high altitude. Age at menarche, total number of pregnancies, and serum testosterone concentrations were greater in women living at high altitude than in those living at sea level. Menopause was attained at an earlier age in women at high altitude than among those living at sea level (P<0·05). Pulse oxygen saturation and serum concentrations of DHEA, DHEAS and androstenedione were lower in samples collected from women living at high altitude. The DHEAS/DHEA ratio was significantly greater, and the androstenedione/testosterone ratio was lower, in samples from women living at high altitude (Table 1).

At sea level and at high altitude, the greatest serum concentrations of DHEA were observed in women between 20 and 29 years of age. Thereafter, a progressive and significant decline in the serum hormone concentrations was observed: at sea level, the value in women aged 60–70 years was 35·8% that at 20–29 years, whereas at high altitude the value at 60–70 years declined to 25% of that at 20–29 years.

In women aged from 20 to 39 years, serum concentrations of DHEA were lower in women living at high altitude than in those resident at sea level. In the group aged 40–49 years, serum concentrations of DHEA were similar in those at sea level and at high altitude (NS). After the age of 50 years, a greater decline in serum concentrations of DHEA was observed in women living at high altitude than in those at sea level, and by 60–70 years of age the serum concentrations among those resident at high altitude were 46·9% of those in women resident at sea level (Table 2).

The greatest concentrations of serum DHEAS were observed among women aged 20–29 years living at sea level, and those aged 30–39 years living at high altitude. Serum concentrations of DHEAS among women aged 30–39 years were similar at both altitudes of residence. The decline in DHEAS at sea level and at high altitude occurred from 40 years of age and was faster at high altitude than at sea level. At 60–70 years, the serum concentration of DHEAS among those living at high altitude was 56% of that at sea level (Table 2).

In women aged 20–29 years, serum concentrations of androstenedione were similar in those living at sea level and at high altitude, whereas in those aged 30–39 years serum concentrations of androstenedione were significantly lower in those resident at high altitude (P<0·005). The difference disappeared at 40–49 years and thereafter was evident again at 50–70 years (Table 2).
The greatest concentrations of androstenedione among women living at sea level and at high altitude were observed at 20–29 years. Serum concentrations of androstenedione among natives of high altitude aged 60–70 years were 27.34% of the value in those living at sea level. In samples from both sea-level and high-altitude groups, serum concentrations of androstenedione declined with age from 20 to 70 years (Table 2).

Among women living at sea level, serum testosterone concentrations did not change with age from 20 to 70 years. In those living at high altitude, the greatest mean values were observed at 20–29 years of age, whereas the values at 60–70 years were 33% lower. In women in age groups 20–39 years and 50–59 years, serum testosterone concentrations were greater at high altitude than at sea level (P<0.05). In those aged 60–70 years, serum testosterone concentrations were similar in women living at sea level or at high altitude (Table 2).

At sea level and at high altitude, the serum testosterone/estradiol ratio increased with age (P<0.0034 and P<0.0001 respectively). This ratio increased earlier in those resident at high altitude (40–49 years) than in women living at sea level (50–59 years). At 60–70 years, there were no differences between the two altitude groups with respect to serum testosterone/estradiol ratios (Table 3).

Multivariate analysis showed that altitude (P<0.0001) and greater chronological age (P<0.001) were associated with lower serum concentrations of DHEAS (Table 4). In Table 5, data are presented showing independent variables associated with serum concentrations of DHEAS, DHEA and androstenedione at high altitude. DHEAS was related to chronological age (P<0.0001). Serum DHEA concentrations were not related to any of the variables included in the equation. Low serum androstenedione concentrations were related to residence at high altitude at birth and greater chronological age (P<0.0001).

### Discussion

Our results demonstrate that adrenopause, the decline in serum adrenal androgens with age, occurred earlier in women resident at high altitude than in those living at sea level. As adrenopause is a marker of somatic aging (Berr et al. 1996, Ravaglia et al. 1996, Labrie et al. 1998), we postulate that somatic aging will also occur early at high altitude.

The greatest concentrations of serum DHEA at sea level and at high altitude were observed in women aged between 20 and 29 years. This is in accordance with data reported in another study (Labrie et al. 1997). In women living at sea level, DHEA values at 60–69 years of age were reduced to 65% of the greatest value at 20–29 years.

### Table 2 Serum concentrations of DHEA, DHEAS, androstenedione and testosterone in women resident at sea level or at high altitude, according to chronological age. Data are means±s.d.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>DHEA (ng/ml)</th>
<th>DHEAS (µg/ml)</th>
<th>Androstenedione (ng/ml)</th>
<th>Testosterone (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sea level</td>
<td>High altitude</td>
<td>Sea level</td>
<td>High altitude</td>
</tr>
<tr>
<td>20–29</td>
<td>3·06 ± 1·42</td>
<td>1·83 ± 0·97**</td>
<td>193·4 ± 89·4</td>
<td>98·8 ± 40·8*</td>
</tr>
<tr>
<td>30–39</td>
<td>1·62 ± 0·56</td>
<td>0·86 ± 0·56*</td>
<td>183·3 ± 79·7</td>
<td>155·5 ± 98·4</td>
</tr>
<tr>
<td>40–49</td>
<td>1·58 ± 0·78</td>
<td>1·39 ± 0·70</td>
<td>103·2 ± 52·9</td>
<td>72·3 ± 28·1*</td>
</tr>
<tr>
<td>50–59</td>
<td>1·16 ± 0·34</td>
<td>0·54 ± 0·42*</td>
<td>100·4 ± 35·9</td>
<td>64·6 ± 35·3*</td>
</tr>
<tr>
<td>60–70</td>
<td>0·98 ± 0·51</td>
<td>0·38 ± 0·29*</td>
<td>98·6 ± 39·1</td>
<td>57·2 ± 36·2*</td>
</tr>
</tbody>
</table>

*P<0·05; **P<0·01 with respect to values at sea level.

### Table 3 Serum testosterone/estradiol ratio in women resident at sea level or at high altitude, according to chronological age. Data are mean±s.d.

<table>
<thead>
<tr>
<th>Testosterone/estradiol ratio</th>
<th>Sea level*</th>
<th>High altitude**</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>2·0 ± 1·0</td>
<td>8·0 ± 28·5</td>
<td>NS</td>
</tr>
<tr>
<td>30–39</td>
<td>1·0 ± 1·2</td>
<td>1·3 ± 1·8</td>
<td>NS</td>
</tr>
<tr>
<td>40–49</td>
<td>1·2 ± 1·7</td>
<td>2·0 ± 46·1</td>
<td>&lt;0·05</td>
</tr>
<tr>
<td>50–59</td>
<td>22·4 ± 24·4</td>
<td>65·2 ± 100·2</td>
<td>&lt;0·05</td>
</tr>
<tr>
<td>60–70</td>
<td>82·0 ± 123·8</td>
<td>50·2 ± 64·8</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P<0·0034; **P<0·0001 with respect to age.

Arrendale et al. (1996, 1997). In women living at sea level, DHEA concentrations in women resident at sea level or at high altitude

<table>
<thead>
<tr>
<th>DHEAS</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place of residence</td>
<td>−7·2 ± 11·3</td>
<td>0·0001</td>
</tr>
<tr>
<td>Chronological age</td>
<td>−2·3 ± 0·70</td>
<td>0·001</td>
</tr>
<tr>
<td>Menopause</td>
<td>−7·6 ± 20·46</td>
<td>NS</td>
</tr>
<tr>
<td>Constant</td>
<td>347·6 ± 25·51</td>
<td>0·0001</td>
</tr>
</tbody>
</table>

Coefficient of determination (R²)=0·38; P<0·00001. N=145. β=Coefficient of regression (mean ± s.d.). Place of residence: 0=sea level; 1=altitude. Menopause: 0=not; 1=yes.
This was similar to findings reported elsewhere in the literature (Labrie et al. 1997). However, in women living at high altitude, the reduction with age was of greater magnitude.

The maximal concentration of DHEAS occurred at later age in women resident at high altitude (30–39 years) than in those living at sea level (20–29 years). This could be related to the delayed presentation of adrenarche and delayed onset of puberty observed in girls living at high altitude compared with those living at sea level (Góñez et al. 1993, Gonzales et al. 1996). Early age at adrenopause could also be related to the early onset of menopause observed at high altitude (Gonzales & Villena 1997). These data suggest that maturation is delayed and aging accelerated in women living at high altitude.

One of the most interesting findings is the increase in serum testosterone concentrations found at high altitude. This seems to be due to a high rate of metabolism from androstenedione to testosterone, and a low rate of metabolism from androstenedione to estradiol. This has also been confirmed in men (Gonzales 1998b). As testosterone is an erythropoietic hormone, high concentrations of hemoglobin observed in the populations of the Andes of Peru may be a consequence of this increase. However, high hemoglobin concentrations may be also associated with chronic mountain sickness (León-Velarde & Arregui 1994), therefore high testosterone concentrations may not afford the adaptive advantage that has been suggested previously (Gonzales 1998b).

In women, most peripheral androgens are produced by the metabolism of DHEA and DHEAS to testosterone and dihydrotestosterone in peripheral intracrine tissues (Phillips 1996, Labrie et al. 1997, 1998). According to our data, differences in serum adrenal androgen concentrations seem to be due to differences in the metabolism of these steroids. Indeed, the DHEAS/DHEA ratio was increased at high altitude.

One of the mechanisms of adrenarche is the reduction in activity of the enzyme 3β-hydroxysteroid dehydrogenase, inhibiting conversion of DHEA to androstenedione. In turn, synthesis and secretion of DHEA are increased (Gell et al. 1998). Activity of 3β-hydroxysteroid dehydrogenase continues to decrease in the zona reticularis of the adrenal cortex in adults (Gell et al. 1998); however, it is unknown what happens during aging. The present study has demonstrated that activity of this enzyme, measured as the DHEA/androstenedione ratio, was not modified with aging, suggesting that mechanisms of adrenarche are different from those of the adrenopause.

The decline in the circulating concentrations of the adrenal androgens with aging seems to be due to a reduction in the mass of the reticularis zone of the adrenal cortex (Parker et al. 1997). However, studies on adrenal cells cultured in vitro demonstrate that the low concentrations of adrenal androgens associated with age seem to be more related to extra-adrenal than to intra-adrenal factors (Fearon et al. 1998). Life at high altitude is characterised by low barometric pressure and low oxygen pressure, and molecular oxygen is an obligatory substrate for the cytochrome P450c17 in the zona reticularis of the adrenal cortex (Roberts 1999).

Differences in serum concentrations of adrenal androgens between samples collected from women resident at sea level or at high altitude could be due to ethnic, smoking or environmental differences. Ethnicity has been demonstrated to affect adrenal androgen concentrations (Hill et al. 1976, Carmina et al. 1992, Kleerekoper et al. 1994, Manson et al. 2001). A recent study has demonstrated that African-American, but not white women had significantly lower concentrations of DHEAS with increasing age (Manson et al. 2001). This does not seem to be the case in the present study. In fact, ethnic composition was similar in women studied at sea level and at high altitude.

Smoking is another factor associated with differences in adrenal androgen concentrations. Several studies have demonstrated greater serum adrenal androgen concentrations in women who are smokers (Cassidenti et al. 1992, Baron et al. 1995, Bancroft & Cawood 1996, Johannes et al. 1999, Laughlin & Barrett–Connor 2000). In the present study, differences in serum adrenal androgen concentrations between women resident at sea level or at high altitude were not attributable to smoking, because fewer than 1% of women from our sample were smokers.

<table>
<thead>
<tr>
<th>Table 5 Multivariate analysis to explain serum DHEA, DHEAS and androstenedione concentrations in women resident at high altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DHEAS</strong></td>
</tr>
<tr>
<td>Altitude at birth</td>
</tr>
<tr>
<td>Years of residence</td>
</tr>
<tr>
<td>Chronological age</td>
</tr>
<tr>
<td>Constant</td>
</tr>
<tr>
<td>$R^2$</td>
</tr>
<tr>
<td>$p$</td>
</tr>
</tbody>
</table>

Data represent coefficient of regression ± s.e. $R^2$, coefficient of determination. N=77. Altitude at birth: 3000–4246 m. *P<0·0001.
The finding that women at high altitude aged 60–70 years had serum concentrations of DHEA and DHEAS that were 50% of the value in those living at sea level suggests a major significance of aging at high altitude. This may explain the greater incidence of symptoms of disease among women aged over 65 years at high altitude than among those resident at sea level (Peru 1997, Gonzales 1998a), and the lower life expectancy among women at Cerro de Pasco (4340 m) than among women resident at sea level (León-Velarde & Arregui 1994).

In summary, our findings show that changes in adrenal androgens with age seem to be associated with processes of maturation and aging. Serum concentrations were greatest in women aged 20–29 years, and then declined to low values after the age of 50 years. The early decline in adrenal androgen concentrations in women living at high altitude may suggest that aging is attained earlier at high altitude. Furthermore, serum adrenal androgen concentrations are associated with the well-being of aging persons (Ravaglia et al. 1996). The observation that, in the same age range (60–70 years), serum adrenal androgens in women living at high altitude were 50% of the values in those resident at sea level may therefore also suggest that greater aging processes operate at high altitude. This may be associated with both the greater prevalence of symptoms of disease in older women and the lower life expectancy at high altitude (León-Velarde & Arregui 1994, Gonzales 1998a).

Acknowledgements

The authors thank Rosario Tapia and Amanda Córdova for their help in the field work. This study was partially supported by the Human Reproduction Programme of the World Health Organisation (WHO) and the Consejo Nacional de Ciencia y Tecnología (CONCYTEC) del Perú, and by the Instituto de Investigaciones de la Altura of the Universidad Peruana Cayetano Heredia.

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Received 11 December 2001
Accepted 14 January 2002