Effect of *Lepidium meyenii* (Maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m)

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Abstract

Lepidium meyenii (Maca) is a Peruvian hypocotyl that grows exclusively between 4000 and 4500 m in the central Andes. Maca is traditionally employed in the Andean region for its supposed fertility-enhancing properties.

The aim of this study was to test the hypothesis that Maca can prevent high altitude-induced testicular disturbances. Adult male rats were exposed for 21 days to an altitude of 4340 m and treated with vehicle or aqueous extract of Maca (666·6 mg/day). The lengths of the stages of the seminiferous epithelium and epididymal sperm counts were obtained at 0, 7, 14 and 21 days of exposure. The stages of the seminiferous tubules were assessed by transillumination. A dose–response study was also performed at sea level to determine the effect of Maca given to male rats at doses of 0, 6·6, 66·6 and 666·6 mg/day for 7 days on body weight, seminiferous tubule stages and epididymal sperm count. The length of stage VIII and the epididymal sperm count were increased in a dose-dependent manner in Maca-treated rats but treatment reduced the length of stage I. At the highest dose, sperm count increased 1·58 times, the length of stage VIII increased 2·4 times and the length of stage I was reduced 0·48 times compared with the value at dose 0. Exposure to high altitude resulted in a reduction in epididymal sperm count after 7 days and lower values were maintained up to 21 days. Altitude reduced spermiation (stage VIII) to half and the onset of spermatogenesis (stages IX–XI) to a quarter on days 7 and 14 but treatment with Maca (666·6 mg/day) prevented these changes. Data on transillumination and epididymal sperm count in the Maca-treated group exposed to high altitude were similar to those obtained at sea level. Maca increased the sperm count on day 21 of exposure to high altitude to values similar ($1095{\cdot}25 \pm 20{\cdot}41 \times 10^6$ sperm, means ± S.E.M.) to those obtained in the Maca-treated group at sea level ($1132{\cdot}30 \pm 172{\cdot}95 \times 10^6$ sperm). Furthermore, in the Maca-treated group exposed for 21 days to high altitude, epididymal sperm count was higher than in the non-treated group at sea level ($690{\cdot}49 \pm 43{\cdot}67 \times 10^6$ sperm).

In conclusion, treatment of rats with Maca at high altitude prevented high altitude-induced spermatogenic disruption.

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Introduction

Being exposed to high altitude means living under conditions of hypoxia, cold, aridity and high ultraviolet radiation. Exposure to high altitude (4340 m) results in testicular disturbances in men (Donayre 1968) and rats (Gonzales *et al.* 1990). This was also observed during exposure to a simulated altitude of 4411 m (Saxena 1995), and also by the effects of cold (Blanco-Rodríguez & Martinez-Garcia 1997). The changes included degeneration of the germinal epithelium and spermatogenic arrest (Gonzales *et al.* 1990, Saxena 1995).

Exposure of male rats to high altitude (4340 m) reduces body weight 3 days after their arrival. This reduction seems also to be due to increased serotonergic activity (Gonzales 1993).

Maca (*Lepidium meyenii*) is a Peruvian hypocotyl belonging to the Brassicaceae family and grows exclusively between 4000 and 4500 m altitude in the central Peruvian Andes. The root, known as Maca, has been used traditionally by Peruvians living at high altitudes as a nutrient, an energizer and for aphrodisiac and/or fertility-enhancing properties. According to folk belief, Maca is a plant that enhances fertility in human and domestic animals, which tends to be reduced at higher altitudes (León 1964). The biological activity of the plant is located in the root. The first evidence that Maca improved spermatogenesis was reported in male rats by Gonzales

et al. (2001*a*) who found that oral administration to normal adult male rats of an aqueous extract from the roots of *L. meyenii* (Maca) for 14 days had a beneficial effect on spermatogenesis, acting on first mitosis (stages IX–XI). Thereafter, Gonzales *et al.* (2001*b*) demonstrated that Maca also improved sperm count and sperm motility in normal men without affecting serum testosterone, luteinizing hormone and follicle-stimulating hormone levels. Its nutritional capability has also been described in mice (Canales *et al.* 2000).

The present study was designed to determine whether treatment with aqueous extracts of Maca to adult male rats exposed to high altitude may prevent the deleterious effect of altitude on spermatogenesis.

Materials and Methods

Animals

Three-month-old male rats of the Holtzman strain obtained from the animal house of the Universidad Peruana Cayetano Heredia were used for the high-altitude exposure study. Rats were divided at random into four groups, two treated and two untreated with Maca. Two groups were maintained at sea level (treated with Maca and untreated) and two groups were kept at high altitude (one treated with Maca and the second untreated). These two groups of rats were transported by bus from Lima at 150 m to Cerro de Pasco at 4340 m. The trip lasted 8 h. For the dose–response study, 75-day-old rats of the Holtzman strain were used.

Rats were housed four to six per cage, maintained at environmental temperature (22 °C at sea level and 10 °C at high altitude) under a 12 h light:12 h darkness cycle and provided with Purina laboratory chow and tap water *ad libitum*.

Experimental protocols

Effect of altitude Rats were randomly assigned to four groups, two of them at sea level and two exposed to high altitude (4340 m). In both locations, one group was treated with Maca (666·6 mg/day) and the second with vehicle. These four groups was subdivided into three different groups of six animals according to the length of treatment (at 4340 m): 7, 14 and 21 days. In the group exposed to high altitude, Maca (666·6 mg/day) was administered 8–12 h after the mice were received at an altitude of 4340 m.

Dose–response study Four male rats per group received 0, 6·6, 66·6 or 666·6 mg aqueous extract of Maca each day for 7 days. The procedure and schedule for administration was similar to that in the altitude experiment. This experiment was performed at sea level.

Treatment To administer Maca or vehicle, an intubation needle no. 18 (Fisher Scientific, Pittsburgh, PN, USA) for nasogastric feeding was used to give, once a day, 2 ml water (with or without Maca) for 7, 14 or 21 days. The rats at both locations were killed on days 7, 14 and 21 of treatment by decapitation and blood was collected. The Institutional Review Board of the Scientific Research Office from the Universidad Peruana Cayetano Heredia approved the study.

Preparation of aqueous extract of L. meyenii *(Maca)*

The root of *L. meyenii* was obtained in Carhuamayo at an altitude of 4000 m. The identity of the plant was authenticated by visual verification by Irma Fernandez, a botanist from the Department of Biochemistry, Molecular Biology and Pharmacology, Universidad Peruana Cayetano Heredia. An aqueous extract of the root was prepared according to the traditional method. In brief, 500 g dried root was placed in a container with 1500 ml water, pulverized and boiled for 30 min. The preparation was left standing to cool and then filtered. The filtrate, containing 333 mg root/ml was placed in small vials and kept in a refrigerator at 4 °C until use.

For the dose–response study, the 333 mg root/ml was diluted 1/10 to obtain a concentration of 33·3 mg/ml. This was further diluted 1/10 to obtain a concentration of 3·33 mg/ml. Rats received 0, 6·66, 66·6 or 666·6 mg Maca/day for 7 days.

Assessment of the stages of the rat seminiferous cycle

Assessment of the length of the stages was made by transillumination under an inverted stereomicroscope at 40 × magnification as previously described (Gonzales *et al.* 2001*a*). A total length of 1000 mm was assessed for each rat. The stages assessed were as follows: I, II–III, IV–V, VI, VII, VIII, IX–XI, XII and XIII–XIV as described originally by Parvinen (1982).

Epididymal sperm count

Homogenization-resistant epididymal sperm from non-perfused rats were counted as described previously (Robb *et al.* 1978) with some modifications. Homogenization was performed in 5 ml saline (NaCl, 0·9%). Modifications included refrigeration of the homogenized epididymal preparation at 4 °C for 24 h to allow sperm to be released from the walls. Data are given as sperm/epididymis.

Serum testosterone levels

Serum testosterone levels were determined by RIA using ^{125}I-testosterone as the radioactive marker. The assay was performed using a commercial kit (Diagnostic Products Co, Los Angeles, CA, USA). All samples were run in the

Table 1 Dose–response effect of Maca on body weight and epididymal sperm count after 7 days treatment. Data are means ± S.E.M. of four rats per group

	Increase in body weight (g)	**Epididymal sperm count** (10^6)
Dose of Maca (mg/day)		
0	20·76 ± 2·35	578·91 ± 2·77
6·66	22·50 ± 0·90	618·87 ± 14·47**
66·6	35·33 ± 3·04*	674·26 ± 2·56**
666·6	33·25 ± 3·17**	916·57 ± 7·08*

*$P<0.01$, **$P<0.05$ compared with values at dose 0. $r^2=0.87$ (y=610·76+0·44x) for epididymal sperm count vs Maca dose correlation.

same assay period. The within-assay variation was 5·5% and sensitivity was 4·0 pg/ml.

Statistical analysis

Data were analyzed using statistical package STATA (version 7·0) for the personal computer (Stata Corporation, College Station, TX, USA). Data are presented as means ± S.E.M. Homogeneity of variances was assessed by the Bartlett test. If variances were homogeneous, differences between groups over time were assessed by two-way ANOVA. Differences between pairs of means were assessed by the Scheffé test. If variances were not homogeneous, the Kruskal–Wallis test was used to assess differences between groups, and the Mann–Whitney test to assess differences between pairs in each group. Multivariate analysis was performed to assess the independent effects of Maca and altitude on epididymal sperm count. A value of $P<0.05$ was considered to be statistically significant.

Results

Dose–response study

Table 1 shows data on body weight increase and epididymal sperm count in male rats treated with 0, 6·6, 66·6 or 666·6 mg Maca/day for 7 days. The increase in body weight from day 0 to day 7 was significantly higher at 66·6 ($P<0.01$) and 666·6 mg Maca/day ($P<0.05$) compared with 0 or 6·66 mg Maca/day. However, when mean body weight on day 7 was compared no differences were observed between the different doses of Maca (0, 6·66, 66·6, 666·6 mg Maca/day). Epididymal sperm count was significantly increased at all doses of Maca with a significant dose–response effect ($r^2=0.87$; y=610·76+0·44x). The highest effect on epididymal sperm count was observed at 666·6 mg Maca/day.

Data on the lengths of stages I and VIII of the seminiferous epithelium cycle where a dose–response effect was observed are shown in Fig. 1. The length of stage VIII increased as the dose of Maca increased. In parallel, a reduction in stage I was observed. Stage IX–XI was not modified at any of the doses of Maca (*P*, not significant (NS)).

Maca and exposure to high altitude

Table 2 shows the body weight changes at 7, 14 and 21 days of exposure. Compared with the untreated group at sea level, body weight was significantly reduced at high altitude up to 14 days ($P<0.01$) and then recovered (*P,* NS).

Body weight increased in the group treated with Maca at high altitude after 7 ($P<0.01$), 14 ($P<0.01$) and 21 days ($P<0.05$) of exposure; significantly higher than in the untreated group at high altitude.

A reduction in serum testosterone levels on days 14 ($P<0.01$) and 21 ($P<0.01$) was observed in male rats treated at sea level with vehicle when compared with day 7. This reduction was not observed in the group treated with Maca (*P*, NS) (Table 3).

In the non-treated group at sea level, serum testosterone levels decreased on days 14 ($P<0.01$) and 21 ($P<0.01$) compared with values on day 7. This reduction was not observed in the Maca-treated group (*P*, NS). However, serum testosterone levels in the Maca-treated group at sea level were higher on day 14 ($P<0.05$) than in the untreated group (Table 3). Serum testosterone levels were similar in both non-treated groups at sea level and at high altitude (*P*, NS). In the untreated group, serum testosterone levels were reduced on day 21 at high altitude ($P<0.05$) compared with values on day 7. In the Maca-treated group, serum testosterone levels after 7 days at high altitude were higher than in the non-treated group at high altitude (Table 3).

Figure 2 shows data related to the lengths of the stages of the seminiferous tubules measured by transillumination in male rats at sea level and in those exposed acutely at high altitude without or with treatment with Maca on days 7 (Fig. 2a), 14 (Fig. 2b) or 21 (Fig. 2c).

Comparing data between untreated rats at sea level and at high altitude, the lengths of stages VIII and IX–XI were significantly reduced after 7 and 14 days (Fig. 2a and b).

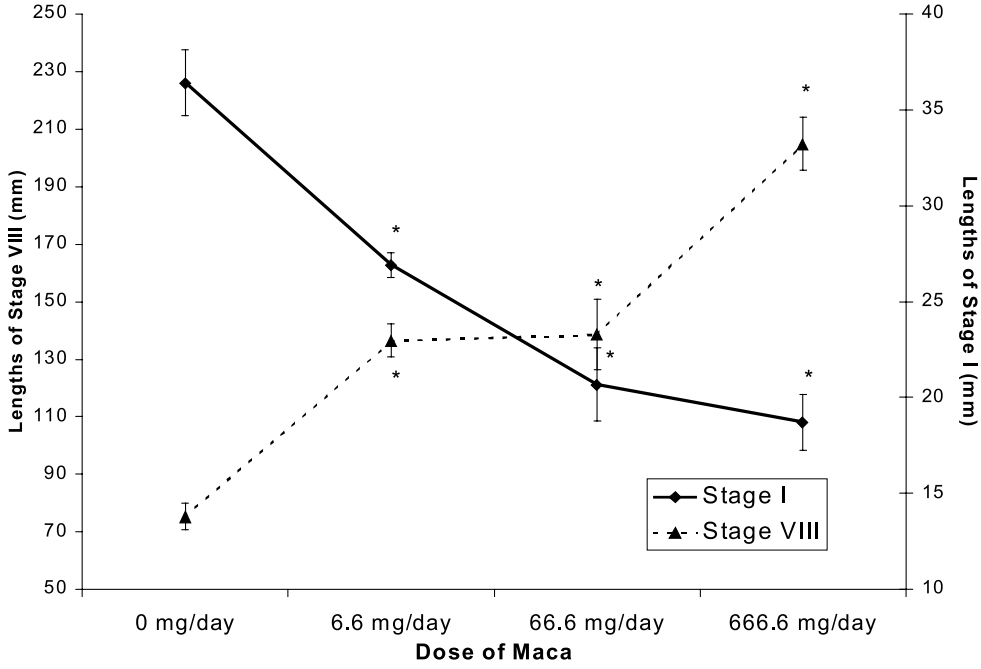

Figure 1 Dose–response curve of the lengths of stages I and VIII of the seminiferous tubules after 7 days of treatment with 0, 6·6, 66·6, or 666·6 mg Maca/day. Data are means ± S.E.M. *$P<0·01$ compared with values at dose 0. Stage I: $r^2=0·34$; y=169·12 − 0·09x. Stage VIII: $r^2=0·65$; y=19·96 0·02x.

At 21 days, untreated rats at high altitude showed stage VIII lengths that were six times higher than at sea level.

At sea level, treatment for 7 days with 666·6 mg Maca/day resulted in increased lengths of stages IV–VI ($P<0·01$) and VIII ($P<0·001$) with a significant reduction in the length of stage I ($P<0·01$). The length of stage VIII after treatment with Maca was 6·7 times higher than in the control group. The length of the stages of the onset of spermatogenesis (IX–XI) was not affected by treatment with Maca for 7 days (Fig. 2a). At day 14, Maca treatment significantly increased stages VI ($P<0·05$), VII ($P<0·01$) and IX–XI ($P<0·01$) (Fig. 2b). At day 21, Maca treatment significantly increased stages II–V ($P<0·05$), VIII ($P<0·01$) and XIII–XIV ($P<0·01$) (Fig. 2c).

At high altitude, treatment for 7 days with 666·6 mg Maca/day resulted in a significant increase in the lengths of stages VI–XII. The highest increase was observed at stage VIII (six times compared with control value). At stage IX–XI, Maca prevented the effect of high altitude. In fact, exposure of untreated rats to high altitude for 7 days resulted in a lower length of stage IX–XI than in Maca-treated rats at high altitude ($P<0·01$) (Fig. 2a). At day 14, Maca treatment significantly increased stages VI, VII, VIII and IX–XI (Fig. 2b). At day 21, treatment significantly increased stages VI and VII ($P<0·01$) (Fig. 2c).

Exposure to high altitude resulted in a reduction in epididymal sperm count at 7, 14 and 21 days. This effect

Table 2 Body weight changes in male rats at sea level and during exposure to high altitude (4340 m): effects of Maca. Data are means ± S.E.M.

	Sea level		**High altitude**	
	Control	Maca-treated	Control	Maca-treated
Days				
0–7	27·17 ± 1·14	25·00 ± 4·12	− 18·89 ± 4·77[a]	57·75 ± 8·56*
0–14	52·83 ± 13·11	31·00 ± 3·13	− 37·83 ± 8·18[a]	54·40 ± 7·55*
0–21	73·33 ± 14·91	49·25 ± 7·58	40·72 ± 14·41	79·33 ± 12·74**

*$P<0·01$, **$P<0·05$ compared with values at high altitude. [a]$P<0·01$ compared with control values at sea level.

Table 3 Serum testosterone levels (ng/ml) in male rats treated or untreated with Maca (666·6 mg/day) at sea level and during acute exposure to high altitude. Data are means ± S.E.M. with the number of rats shown in parentheses

	Sea level		High altitude	
	Control	Maca-treated	Control	Maca-treated
Day				
7	2·13 ± 0·31 (10)	2·86 ± 0·77 (10)	1·57 ± 0·46 (6)	4·60 ± 1·07** (6)
14	0·92 ± 0·20[d] (6)	3·36 ± 0·90[a] (6)	0·87 ± 0·46 (6)	0·78 ± 0·34[b,d] (6)
21	0·88 ± 0·17[d] (6)	1·77 ± 0·72 (6)	0·48 ± 0·42[c] (6)	1·03 ± 0·19[d] (6)

**$P<0·05$ compared with control values at high altitude. [a]$P<0·05$ compared with control values at sea level. [b]$P<0·05$ compared with the Maca-treated group at sea level. [c]$P<0·05$, [d]$P<0·01$ compared with values on day 7. Data were assessed by the Kruskal–Wallis test to assess differences between times, and with the Mann–Whitney test to assess differences between pairs in each group (between times or between control and Maca-treated groups).

was not observed in male rats treated with Maca (Table 4). At day 21 of exposure to high altitude, epididymal sperm counts in male rats treated with Maca were higher than in untreated rats at sea level ($P<0·01$) and similar to rats treated with Maca at sea level (*P*, NS).

Table 5 shows multiple regression analysis in which altitude negatively affected epididymal sperm count whereas it was positively affected by treatment with Maca ($r^2=0·42$, $P<0·0001$).

Discussion

The present study was aimed at demonstrating whether Maca, as traditionally prepared, might prevent high altitude-induced testicular disturbances. It is known that exposure to high altitude results in damage to spermatogenesis in humans (Donayre 1968) and other animals (Gonzales *et al.* 1990, Saxena 1995). The present study demonstrated that exposure of male rats to an altitude of 4340 m for a period of 21 days resulted in low epididymal sperm count. The length of stage VIII was significantly reduced on days 7 and 14 of exposure. This shorter length of stage VIII will result in the lower epididymal sperm count observed on days 7 and 14 of exposure to high altitude.

Spermatogenic arrest induced by high altitude may be due to an increased serotonergic activity (Gonzales *et al.* 1990). Furthermore, serotonin has been demonstrated to affect spermatogenesis by acting directly on the testis (Hedger *et al.* 1995), and it has been shown that hypoxia may increase serotonin levels (Awabdy *et al.* 2003). Serotonin may produce dietary imbalance (Silva *et al.* 2003). It is known that diet restriction may affect spermatogenesis and sperm number in male rats (Brinkworth *et al.* 1992). It is therefore possible that dietary imbalance may affect spermatogenesis during exposure to high altitudes.

At a dose of 666·6 mg/day, Maca has been demonstrated to increase spermatogenesis in male rats, acting on stages IX–XI after 14 days of treatment (Gonzales *et al.* 2001*a*). In the present study, data were obtained on the effect of Maca on spermatogenesis on days 7, 14 and 21. According to the results of this study, one of the first effects of Maca is on stage VIII where spermiation occurs. In fact, after 7 days of treatment, a significant increase in the length of stage VIII occurred. This increase in the length of stage VIII may explain the increase in epididymal sperm count after 7 days of treatment. The increase in the length of stage VIII was accompanied by a reduction in stage I and an increase in the lengths of stages IV–VI. Final maturation of spermatids occurs from stages I to VII (Kangasniemi *et al.* 1990) and this may suggest that Maca could act by stimulating the progression of spermatogenesis from stage I to stage VIII where release of spermatozoa to the lumen of the seminiferous tubules occurs. This may increase the amount of spermatozoa in the epididymis as early as day 7 of treatment.

According to our previous report (Gonzales *et al.* 2001*a*) on the effect of Maca on spermatogenesis in rats, a dose of 666·6 mg/day in a rat of about 300 g body weight represents 2·2 g/kg. If we assume that the average weight of a man is 77 kg, this would translate to about 200 g Maca/day. This amount would be eaten if a fertility-enhancing property is required.

The dose–response study on the effect of Maca on epididymal sperm count shows that a better effect was observed with a dose of 666·6 mg/day (2·2 g/kg). We have previously demonstrated that gelatinized Maca, a pharmaceutical product, at a dose of 1·5–3·0 g/day for 4 months may improve sperm count in men but this effect was observed in only five out of nine men (Gonzales *et al.* 2001*b*). All of this suggests that the doses prescribed by pharmaceutical laboratories to increase sperm count are too low for optimal effect.

The present study demonstrated that Maca (666·6 mg/day) administered to male rats exposed to high altitude prevented the reduced body weight and epididymal sperm count induced by high altitude. Body weight in the

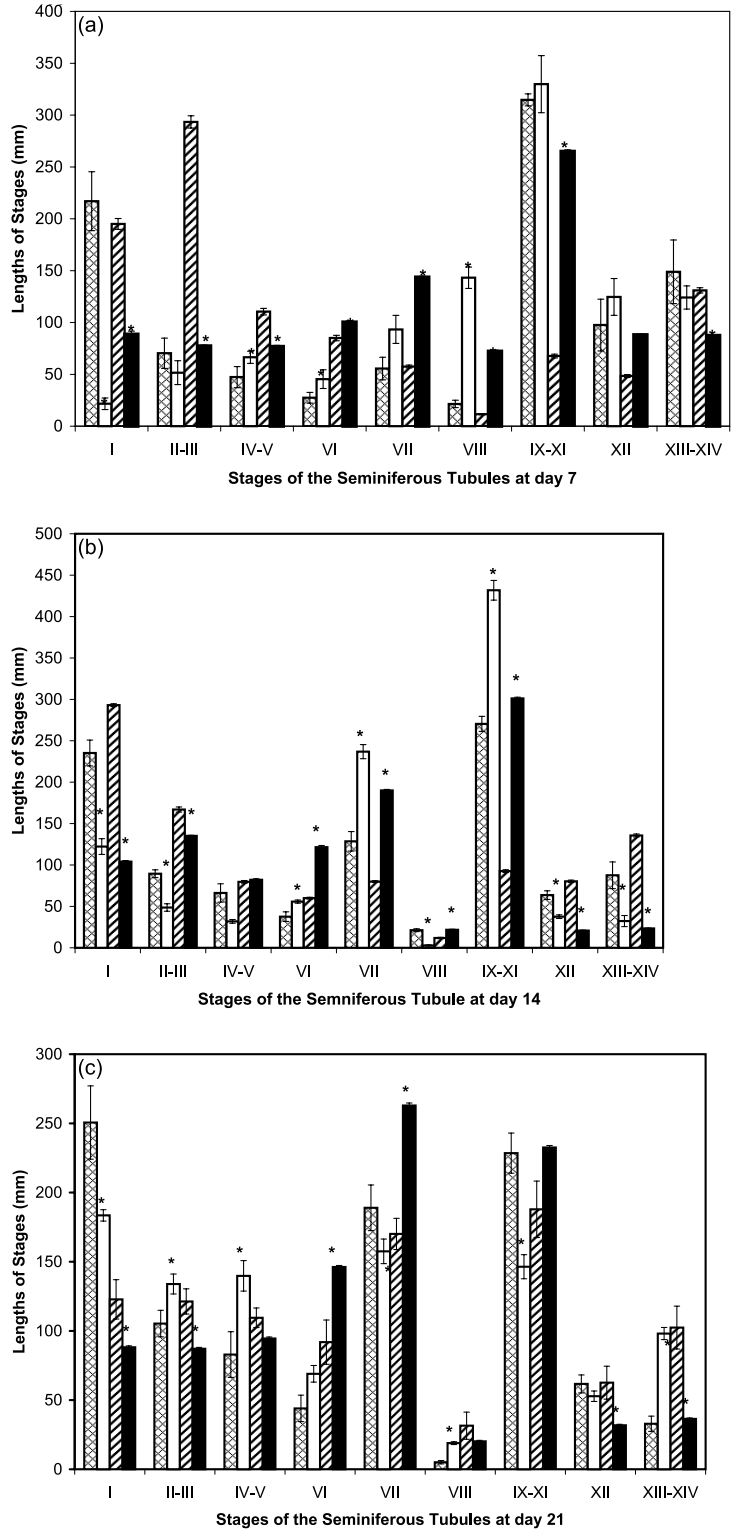

Figure 2 The lengths of stages of the seminiferous tubules on (a) day 7, (b) day 14 and (c) day 21 in untreated male rats at sea level (cross-hatched bars; first bar in each group of four bars), Maca-treated rats at sea level (open bars; second bar in each group), untreated rats at high altitude (hatched bars; third bar) and Maca-treated rats at high altitude (solid bars; fourth bar). Maca was given at a dose of 666·6 mg per day. Data are means ± S.E.M. $^{*}P < 0{\cdot}01$, $^{**}P < 0{\cdot}05$ compared with data from the control group.

Table 4 Epididymal sperm count (10^6 sperm) in male rats exposed acutely to 4340 m altitude and either untreated (control group) or treated with an aqueous extract of Maca (Maca-treated group). Data are means ± S.E.M. with the number of rats shown in parentheses

	Sea level		High altitude	
	Control	Maca-treated	Control	Maca-treated
Day				
7	898·75 ± 27·57 (6)	1315·51 ± 116·55* (6)	402·26 ± 80·43[a] (6)	783·99 ± 33·30*,[c] (6)
14	773·52 ± 16·78[d] (6)	630·17 ± 25·43*,[d]	394·61 ± 85·39[a] (6)	863·32 ± 20·03*,[c] (6)
21	690·49 ± 43·67[d] (5)	1132·30 ± 172·95** (6)	410·72 ± 83·68[b] (6)	1095·25 ± 20·41*,[d] (6)

*$P<0·01$, **$P<0·05$ compared with control values at high altitude. [a]$P<0·05$, [b]$P<0·05$ compared with control values at sea level. [c]$P<0·01$ compared with the Maca-treated group at sea level. [d]$P<0·01$ compared with values at day 7.

Maca-treated animals at high altitude was similar to that at sea level, suggesting that Maca had prevented the weight loss. Furthermore, at high altitude, Maca had increased epididymal sperm count on day 21. In fact, male rats treated with Maca and exposed to 4340 m showed higher lengths of stage VIII (spermiation) and similar lengths of stages IX–XI (onset of spermatogenesis) and similar epididymal sperm counts to those found at sea level, and values significantly higher than those observed in untreated male rats exposed to high altitude. This suggests that Maca reduced the effects of altitude on spermatogenesis by protecting onset (stages IX–XI) and spermiation (stage VIII). The latter avoids the reduction in epididymal sperm count observed on day 7 of exposure.

The effect of Maca is observed at two levels: one of them at stage VIII and the second at stages IX–XI. Stage VIII is associated with spermiation, i.e. the release of spermatozoa to the lumen of the seminiferous tubules. Maca also acts on stages IX–XI where spermatogenesis begins with first mitosis of spermatogonia A (Parvinen 1982). We have previously demonstrated that *L. meyenii* (Maca) administered for 14 days to normal adult male rats may improve spermatogenesis in spermatogonial mitosis (Gonzales *et al.* 2001*a*). Our results demonstrated, in an experimental model in which damage to spermatogenesis was induced by exposure to high altitude, that Maca might also maintain (days 7 and 21 of exposure) and increase the onset of spermatogenesis (day 14). Our data also showed that Maca may act on the stages of spermiation, as stages VII–VIII were significantly increased during exposure to high altitude. The highest value of the length of stage VII was observed on day 21 of exposure to altitude. This may explain the significantly higher value in epididymal sperm count on day 21 of exposure to high altitude than at sea level in male rats treated with Maca.

Treatment for 7 days with Maca (666·6 mg/day) did not affect serum testosterone levels in male rats. This has also been observed in men treated with 1500 or 3000 mg/day (Gonzales *et al.* 2003). This suggests that the effect of Maca on spermatogenesis, particularly on stages VIII and IX–XI, could be due to a direct effect on the testis.

Vehicle administered by intubation resulted in the reduction of serum testosterone levels on days 14 and 21 compared with data on day 7 in male rats at sea level. This effect could be due to stressful manipulation with the intubation needle during gastric feeding. Immobilization stress affects testosterone levels in animals (McGrady 1984). The reduction in serum testosterone levels over time was not observed in the group treated with Maca at sea level. Tapia *et al.* (2000) reported a reduction in stress in mice fed with diets supplemented with Maca, and therefore reducing stress with Maca may prevent the inhibitory effect of intubation on serum testosterone levels. Gonzales *et al.* (1990) showed that serum testosterone levels in male rats increased after 4 days of exposure to high altitude (4340 m). Mujica (1994) demonstrated that serum testosterone levels were significantly higher at 7, 14

Table 5 Multiple regression analysis to test the probability that altitude and treatment with Maca affect epididymal sperm count in male rats. Data are means ± S.E.M.

	Coefficient of regression	Significance
Treatment with Maca	316·58 ± 60·97	<0·0001
Exposure to altitude	− 327·50 ± 112·01	<0·005
Time of exposure to altitude	79·93 ± 45·73	<0·087
Constant	598·69 ± 151·66	<0·0001

Treatment with Maca: yes or no. Exposure to altitude: yes or no. Time of exposure: 0, 7, 14 and 21 days. Coefficient of determination: $r^2=0·42$, $P<0·0001$.

and 21 days of exposure to an altitude of 4500 m. In our study at high altitude, we observed that serum testosterone levels were similar to values at sea level on days 7, 14 and 21. This may be due to the stress of intubation. Once again, treatment with Maca resulted in higher serum testosterone levels in rats exposed for 7 days to high altitude than in untreated animals.

It is also possible that the stress of transportation and relocation into unfamiliar surroundings may affect serum testosterone levels. In our early observations (Gonzales *et al.* 1990), control animals at sea level were transported for 10 h to new surroundings at sea level. In the present study, we have not controlled for transport because of the previous results indicating that altitude *per se* increases serum testosterone levels. We did not measure serum testosterone levels on day 0, as the study was designed to compare the effect of altitude on Maca and altitude on days 7, 14, and 21 compared with animals at the same times. However, the results raised the possibility of an effect of stress from the intubation procedure and further study will be required to demonstrate this effect.

It is interesting that altitude affected body weight, spermatogenesis and epididymal sperm count, and that Maca prevented all of these three variables. It is therefore probable that the effect of altitude on spermatogenesis might, in part, be mediated by the reduction in body weight or by a factor that reduces both body weight and spermatogenesis. Serotonin has been implicated as a factor that regulates reduction in both body weight and spermatogenesis at high altitude (Gonzales *et al.* 1990, Gonzales 1993). However, reduction in body weight *per se* may affect the reproductive system. In rats, dietary restriction was found to deplete the number of sperm, probably because of a lack of calories and/or non-energetic components of the diet (Brinkworth *et al.* 1992).

It is possible that Maca may be acting on fertility because of its high nutritional value. The nutritional value of dried Maca hypocotyls is high (Li *et al.* 2001). Dry Maca hypocotyls have 59% carbohydrates, 10·2% proteins, 8·5% fiber, 2·2% lipids and a number of other compounds, including most of the essential amino acids (Dini *et al.* 1994). Arginine, a constituent of Maca, has been clinically proven to play a role in male fertility (Scibona *et al.* 1994). Maca also contains sterols, such as campesterol, stigmasterol and β-sitosterol (Zheng *et al.* 2000); however β-sitosterol has been found to be an anti-fertility agent in male rats rather than a compound that enhances fertility (Malini & Vanithakumari 1991). Piacente *et al.* (2002) found that Maca also contained (1R,3S)-1-methyltetrahydro-β-carboline-3 carboxylic acid. β-Carbolines inhibit apoptosis (Park *et al.* 2003), and this may be a mechanism that improves spermatogenesis.

Maca is broadly used in Peru as a nutrient. Our data suggest that this nutrient effect needs to be taken into account to explain the fertility-enhancing effect observed at high altitude.

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