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—Full Paper—

Time Course of Endocrine Changes in the Hypophysis-Gonad Axis Induced by Hypobaric Hypoxia in Male Rats

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Abstract. Chronic hypobaric hypoxia (CHH) induces a decrease in sperm output and spermatogenesis in male rats. The mechanisms that underlie these changes in testicular function are unknown and could involve changes in the hypophysis-gonad axis. We have tested the hypothesis that changes take place in the endocrine status (FSH, follicle stimulating hormone; LH, luteinizing hormone; testosterone) of rats subjected to CHH. Male Wistar rats were maintained under normobaric or hypobaric conditions (428 torr, 4,600 m). On days 0, 5, 15 and 30 post-exposure, 12 rats were anesthetized, their body weights were measured and blood samples were collected. The testicles were fixed in 4% formaldehyde and processed for histological analysis. In this time course, the FSH levels rose by day 5 post-exposure. On subsequent days, the FSH levels decreased in rats subjected to CHH with a tendency to remain higher than the normoxic group. The LH plasma levels decreased in rats exposed to CHH. Consistent with the decrease in LH levels, the plasma testosterone level decreased significantly after 30 days of CHH exposure. Integrated analysis of hormonal changes in rats subjected to CHH and the body dehydration that occurs in HH allows us to conclude that the effects of CHH on spermatogenesis may be partially related to changes in the hypophysis-gonad hormonal axis.
Key words: Fertility, High altitude, Hypobaric hypoxia, Testicular function, Testis

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Chronic hypobaric hypoxia (CHH) is experienced by an increasing number of sea level natives exposed to high altitude because of tourism, border patrol, mining or rural health and education activities [1]. It has been suggested that hypobaric hypoxia reduces fertility in humans. Nonetheless, epidemiological studies of high and low altitude populations have not been able to verify this proposal [2]. Based on the hormonal changes observed in men and rats at high altitude, some authors have proposed that CHH affects the hypothalamic/gonad axis (e.g. [3–5]). However, the results in the literature have been controversial. Nelson *et al*. reported that the plasma levels of GH, LH, FSH and TSH of hypoxic rats did not differ from the control values [3]. These results are in agreement with the reported absence of changes in the testosterone levels of rats exposed to hypoxia compared with sea level controls published by Gonzales *et al*. [5]. Instead, Sawhaney *et al*. [4] reported a decrease in the LH and testosterone levels in men exposed to CHH. Exposure of male rats to chronic hypobaric hypoxia and intermittent hypobaric hypoxia induced evident changes in testicular morphology [6–9], strong metabolic stress and loss of spermatogenic cells [8]. Local changes observed in testicles exposed to hypoxia include neovascularization and an increase in temperature. Thus, local changes that could lead to the observed effects on spermatogenesis do occur. However, it is highly likely that these local changes are also accompanied by changes in the hypophysis-gonad endocrine axis.

In the present study, male rats exposed to CHH decreased their food intake (see Results) and became dehydrated, two facts apparently not considered in previous studies and analysis of endocrine changes under CHH. Low food intake has been shown to be related to infertility and a decreased hypophysis-gonad hormonal axis in animals and men (e.g., [10, 11]). Hence, it is likely that some of the changes (or absence of changes) in endocrine parameters could be partially attributed to the nutritional status of the animals and/or water balance. In this work, we adjusted the food intake of normoxic rats to that of CHH animals in order to have a more standardized design of the involvement of the hypophysis/testis axis in the changes observed in rat spermatogenesis. Our results show that the LH level decreased significantly under CHH but was initially not correlated with the testosterone level. Testosterone decreased significantly only after 30 days of CHH exposure. On the other hand, the FSH level rose transiently five days after CHH exposure and subsequently returning to a normal level.

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Materials and Methods

Experimental design

Ten week old male Wistar rats (251 ± 9 g, n=64) were separated in two groups: the sea level normobaric control (Nx) and chronic hypobaric hypoxia (CHH) groups. Each group consisted of 32 individuals housed in cages with 4 individuals per cage and 12:12 light/dark cycle. The CHH animals were exposed to a 4,600 m

simulated altitude (428 torr; P_O_2: 89.6 mmHg) for a period of 5, 15 and 30 days. Pressure changes in a hypobaric chamber were achieved in steps of 150 m/min in order to simulate altitude changes. In order to normalize the food intake for both groups of animals, the food intake of the Nx rats was adjusted to that of the CHH animals (i.e., 15 g/rat per day). Food intake in rats subjected to CHH and Nx was determinate in rats with characteristics similar to the animals previously mentioned. The Nx animals were housed in the same room and next to the CHH animals (22 ± 2 C, 15 g/ rat per day and 1 litre of water per cage). All procedures were performed in agreement with the Principles of Laboratory Animal Care advocated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone analysis

The plasma levels of FSH, LH and testosterone were measured using the following commercial RIA kits: Coat-A-Count FSH immunoradiometric assay (IRMA), Coat-A-Count LH IRMA and Coat-A-Count Total Testosterone (Diagnostic Products Corp., Los Angeles, CA, USA). The sensitivity of the assay was 0.06 mIU/ml for FSH, 0.15 mIU/ml for LH and 0.15 pg/ml for testosterone. The minimum detection levels were 0.85 mIU/ml for FSH, 0.54 mIU/ml for LH and 0.04 ng/ml for testosterone. The intra- and interassay CVs were <3.0% and 1% for FSH, <5.0% and 2% for LH and <9.0% and 11.0% for testosterone. The human/rat cross-reactivity human/rat was > 60% for FSH, LH and testosterone. Blood was collected into syringes containing heparin by cardiac puncture of the left ventricle of the rats (Nx y CHH), which were anaesthetized with ketamine HCl (50 mg/kg). Blood sampling was performed between 1000 and 1030 h for each group. It is known that FSH pulses are less marked than LH pulses [12].

Statistical analysis

The data obtained under CHH conditions were compared with those obtained under the Nx conditions using an Anova test followed by Bonferroni analysis. Differences were accepted as significant when $P<0.05$. The data was analyzed using the GraphPad Prism v 2.01 software (GraphPad Prism Software, San Diego, CA, USA). The results are presented as mean ± SE.

Results

Food intake of rats subjected to CHH

Table 1 shows that the rats subjected to CHH ingested 50% less food than the Nx rats when both groups had *ad libitum* access to food and water. In order to normalize the food intake for both groups of animals, the food intake of the Nx rats was adjusted to the average of the CHH animals (i.e., 15 g/rat/day). This normalized food intake protocol was utilized in those experiments where FSH, LH and testosterone were measured.

Rats become dehydrated when subjected to CHH

In spite of the fact that the food intake of the Nx rats was adjusted to that of the CHH animals, the mean body mass of the CHH rats was lower (201 ± 2 g) compared with the Nx rats (299 ± 7 g) during the exposure period [9]. When the CHH rats were returned to normobaric conditions (sea level), they drank an abundant amount of water and their body weights increased by 14 ± 2 g on average after 2 hr (Table 1). During this period of time, the rats consumed an average of less than 1 g of food, indicating that at least 7% of the weight recovery was from dehydration-driven water intake compensation. These results indicate that, compared with the Nx rats, an important percentage of the diminished body weights of the CHH rats was due to a dehydration process under CHH conditions. This dehydration process could be responsible for the relatively rapid changes observed in the blood haematocrit values of CHH rats [9] and would certainly affect the plasma or serum concentrations of hormones or metabolites measured in these animals.

Table 1. Effect of hydration on rat body mass after hypobaric hypoxia

Day	Before hydration T_0 (g)	After 1 hr of hydration (g)
5	236 ± 3	248 ± 2*
15	216 ± 1	231 ± 4*
30	201 ± 2	217 ± 3*

Rats subjected to chronic hypobaric hypoxia were returned to normoxic conditions at time=0 (T_0). N=8. Mean ± SE. Statistical analysis: Anova followed by Bonferroni analysis. *P<0.05 *vs.* Nx (control). Nx, sea level (normobaric control).

FSH, LH and testosterone plasma levels under CHH

In order to evaluate and correlate plasma FSH levels with other sex hormones and the extent of the spermatogenic process, the FSH plasma levels were determined at different CHH exposure times. At day 5 post exposure, the plasma FSH level of the CHH group was increased significantly compared with the Nx group (Fig. 1). On subsequent days after exposure, the FSH levels of the CHH group decreased but had a tendency to remain higher than those of the Nx group. The LH plasma levels underwent a decrease in the rats exposed to CHH (Fig. 2). Consistent with the decrease in the LH levels, the plasma testosterone levels showed a tendency to decrease in the CHH rats (Fig. 3).

Discussion

Male rats have previously been demonstrated to respond to CHH with cardiovascular changes similar to mine workers labouring at high altitude [1]. Exposure of male rats to intermittent hypobaric hypoxia and chronic hypobaric hypoxia produces an increase in haematocrit, decrease in testicular mass, deterioration of interstitial cells, increase of the interstitial space, damage to the germinal epithelium, increase of the seminiferous tubule lumen, a strong metabolic stress and loss of spermatogenic cells [8, 9]. The testicles of these rats also show remarkable changes in vascularization and temperature [9]. The mechanisms that underlie these changes in testicular and spermatogenic cell physiology under CHH are unknown and could involve the hypophysis-testicle endocrine status as well as local homeostatic mechanisms. Local changes in the

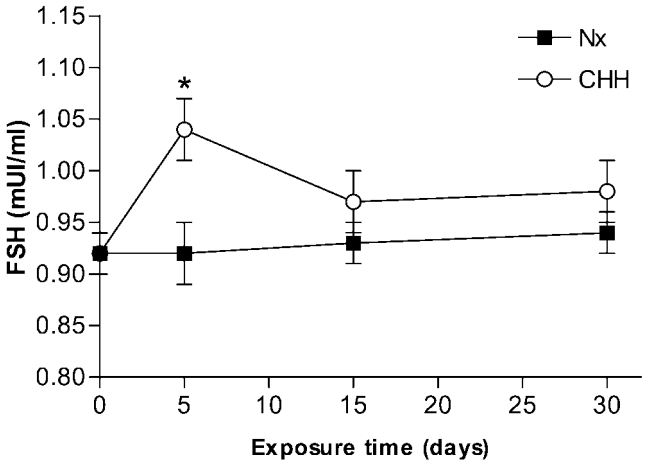

Fig. 1. Effect of chronic hypobaric hypoxia on FSH plasma levels. Mean ± SE. N=8 for each group. Nx, sea level (normobaric control). *P<0.05 *vs*. control.

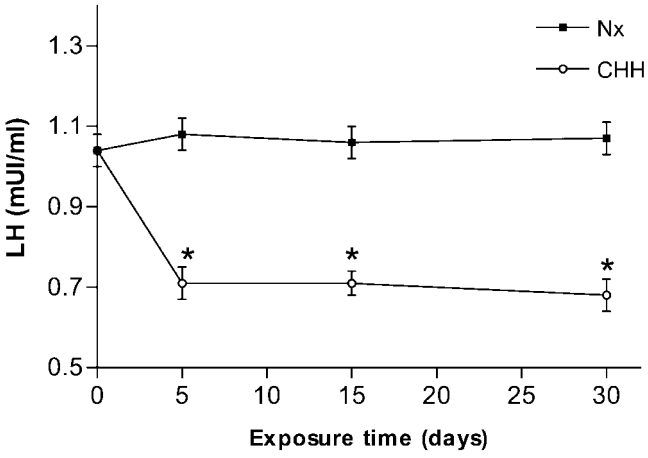

Fig. 2. Effect of chronic hypobaric hypoxia on LH plasma levels. Mean ± SE. N=8 for each group. Nx, sea level (normobaric control). *P<0.05 *vs*. control.

testis, vascularization and temperature changes are well substantiated in our previous work [9]. Thus, we conducted the present study because of divergent results in the literature concerning hormonal changes (gonadotropins and testosterone) under CHH that could potentially be responsible for the changes in testicular histology observed under this condition. In the present study, we considered the changes in nutritional status that occur in animals subjected to CHH and included in our analysis the changes in hydration that take place in these individuals. The male rats exposed to CHH had body weight values that were lower that their Nx counterparts, in spite of the fact that the average food intake was normalized to the average intake of the CHH rats. An important fraction of the decrease of body weight in the CHH rats can be attributed to dehydration (Table 1). Our results show that the FSH level rose transiently under CHH. On the other hand, the LH levels decreased significantly during the same period. The testosterone levels showed a tendency to decrease upon CHH exposure and became significantly lower in the rats at 30 days of CHH. Loss of body water during the period of this study (7–8%) likely implies a relatively homogenous loss of water both from extracellular and intracellular compartments (e.g., [13]). This also implies that it is highly likely that the plasma volume would be decreased and that all blood cellular components and protein or protein bound metabolites would be concentrated in rats exposed to CHH. Although part of the increase in haematocrit could be attributed to a decrease in the plasma volume (7–8%), the changes in haematocrit observed previously [1, 8, 9] should be ascribed to an increase in the number of red blood cell, most likely due to erythropoietin activation of bone marrow in CHH rats (e.g., [14]). The proposed decrease in plasma volume implies that decreases of plasma components would be underestimated, while increases in plasma components could be partly attributed to blood concentration. In this context, the observed effects of CHH on LH (decreases) are likely underestimated by approximately 7%. However, the significant decrease in testosterone level in rats subjected to CHH for 30 days is likely

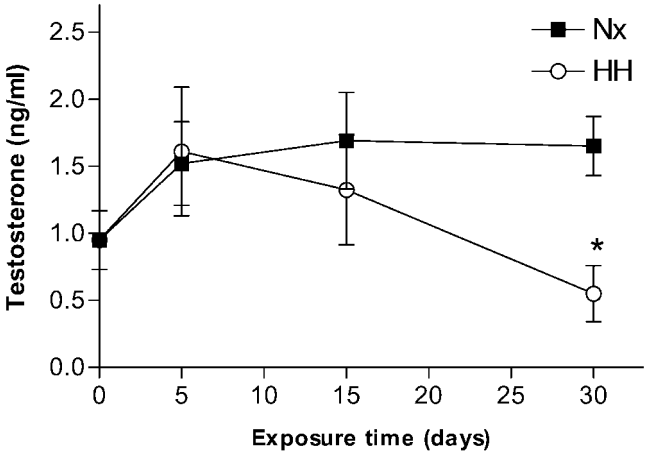

Fig. 3. Effect of chronic hypobaric hypoxia on testosterone plasma levels. Mean ± SE. N=8 for each group. Nx, sea level (normobaric control). *P<0.05 *vs*. control.

underestimated because of the dehydrated condition of the rats, but it may represent the true changes in the endocrine status of these animals. Increases or decreases in body weight, hematocrit and water intake-related changes in body weight followed by stabilization appear to be a function of time for each of these parameters. On the other hand, the FSH level appears to be transiently higher in CHH rats. The FSH and LH plasma levels are related to both the hypothalamus/hypophysis status and the germinal epithelium physiological state, which feeds back to the hypothalamus/hypophysis through inhibin B (FSH) or testosterone production (LH). It has been shown previously that a strong inverse correlation exists between inhibin B and FSH in relation to spermatogenic damage [15]. Hence, a possible interpretation of the FSH increase at 5 days is that these changes were due to both decreased spermatogenesis

(decreased inhibin B negative feedback on the hypothalamus/hypophysis) and likely a dehydration-derived plasma concentration. However, the rise in FSH level (14% in average) cannot be accounted by a decrease in plasma volume only (7%). The return of FSH toward control values can be interpreted as a decrease in hypothalamus-hypophysis activity under hypoxia exposure. Our results showing a continuous decrease in the LH level under CHH exposure are in agreement with this effect of hypoxia on the activity of the hypothalamus-hypophysis from 5 days onward. There was a significant decrease in the testosterone level after 30 days of CHH exposure; its changes lagged behind those of the LH level, as expected and in agreement with the changes in Leydig cell volume after CHH exposure as reported by Gosney, 1984 [16]. Thus, our data indicate that, after normalizing the food intake of the CHH and Nx rats, there are significant changes in the hipophysis-gonad endocrine axis under CHH. This effect of CHH on the hypophysis-gonad axis is strongly suggested by the sustained decrease in LH induced by hypoxia. The changes in FSH likely occur first because of local testicular changes and decreased spermatogenesis and inhibin feedback. Thus, the height of the spermatogenic epithelium in the CHH rats decreased significantly (52 ± 9 μm) compared with the Nx rats (98 ± 11 μm) [9]. These early local effects of CHH on spermatogenesis have been previously substantiated [8, 9]. The changes in this parameter strongly suggest that CHH rats have decreased proliferation of the male germinal epithelium and premature release of spermatids and that the hypoxic protocol effectively affected spermatogenesis. The return of FSH toward normal levels and the decrease in the LH plasma level can be explained as a decrease in the activity of the hypothalamus-hypophysis axis. In this rat model, it is likely that the long term effects of hypoxia on testicular physiology and spermatogenesis, especially on spermiogenesis, can be ascribed to a decrease in the testosterone level. In conclusion, the effects of CHH on spermatogenesis may be partially related to changes in the hypophysis-gonad hormonal axis, as FSH rises initially due to spermatogenic damage and inhibin B altered feedback but recovers afterwards, being relatively low (as LH is) at longer periods, as found also for testosterone.

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