HIGH ALTITUDE MEDICINE & BIOLOGY
Volume 15, Number 3, 2014

DOI: 10.1089/ham.2014.1022

Gestational and Early Postnatal Exposure to Simulated High Altitude Does Not Modify Postnatal Body Mass Growth Trajectory in the Rat

Carlos E. Bozzini, Graciela M. Champin, Clarisa Bozzini, and Rosa M. Alippi

Abstract

Bozzini, Carlos E, Graciela M. Champin, Clarisa Bozzini, and Rosa M. Alippi. Gestational and Early Postnatal Exposure to Simulated High Altitude Does Not Modify Postnatal Body Mass Growth Trajectory in the Rat. *High Alt Med Biol* 15:418–421, 2014.—Postnatal hypoxia blunts body mass growth. It is also known that the quality of the fetal environment can influence the subsequent adult phenotype. The main purpose of the study was to determine whether gestational hypoxia and early postnatal hypoxia are able to blunt growth when the offspring is raised under normoxia. Hypobaric hypoxia was induced in simulated high altitude (SHA) chambers in which air was maintained at 380 mmHg (5450 m). Mature Sprague-Dawley rats of both sexes were divided in *normoxic (NX)* and *hypoxic (HX)* groups and, in the case of the HX group, maintained for 1 month at 5450 m. Mating was then allowed under NX or HX conditions. Offspring were NX-NX, NX-HX, HX-HX, or HX-NX: the first term indicates NX or HX during both gestation and the first 30 days of life; the second term indicates NX or HX during postnatal life between days 30 and 133. Body mass (g) was measured periodically and body mass growth rate (BMGR, g/d) was estimated between days 33 and 65 of postnatal life. Results can be summarized as follows: 1) BM was significantly higher in NX than in HX rats at weaning; 2) BMGR was not significantly different between NX-NX and HX-NX rats, and between HX-HX and NX-HX animals; and 3) BMGR was significantly higher in rats living under NX conditions than in those living under HX conditions during postnatal life. Data suggest that that hypobaric hypoxia during gestational and early postnatal development of rats does not alter the regulation of body mass growth in rats when compared to that seen under sea-level conditions.

Key Words: body mass; developmental phenotype; growth; hypoxia; simulated high altitude

Introduction

Each animal species shows a characteristic growth trajectory that can be altered in rate and timing by exogenous modifiers. Altitude hypoxia is an important environmental factor that is able to influence the growth pattern (Delaquierre-Richardson et al., 1965; Schnakenberg et al., 1971; Alippi et al., 1983; Bozzini et al., 1998; Schwartz et al., 1998; Morel et al., 2005; Farahani et al., 2008). Under hypoxia, body growth rate is depressed in growing experimental animals. As a consequence, the body mass is reduced for the chronological age and gender (Timiras et al., 1957; Delaquierre-Richardson et al., 1965; Alippi et al., 1983; Mortola et al., 2000; Bozzini et al., 2013). The effect of hypoxia on body growth rate has been associated with hypophagia because of reduced appetite (Alippi et al., 1983; Elia et al., 1985; Westerterp-Plantenga et al., 1999; Morel et al, 2005), which suggests that the hypoxic animals grow in accordance to the amount of food consumed. This thought is supported by several findings: 1) a subnormal food intake in relation to metabolic body weight (appetite quotient) and a normal utilization of dietary protein for growth in infantile rats exposed to simulated high altitude (SHA) (Alippi et al., 1983); 2) a similar body mass loss in fasted rats either exposed for 48 h to SHA or maintained under sea level conditions (Schnakenberg et al., 1971; Norese et al., 2002); and 3) a similar loss of body mass in normoxic and hypoxic rats that were pair-fed (Norese et al., 2002).

Department of Physiology, Faculty of Odontology, University of Buenos Aires, Argentina.

Indirect evidence that growth retardation during exposure to SHA could be due to resetting of a putative central control (set-point) with a mechanism for setting target body mass size has been also presented (Bozzini et al., 2005). If we accept the above hypothesis, it could be inferred that hypoxia, by reducing appetite and thus energy intake, could operate on the phenotype of the individual to reduce body mass, which may be an important physiological response in the process of acclimation to high altitude by reducing oxygen requirements (Monge and León-Velarde, 1991; Mortola, 1993).

It is now well recognized that the quality of the fetal environment can profoundly influence the subsequent adult phenotype (Barker, 1994). According to the developmental origins of health and disease hypothesis, *in utero* experiences reprogram an individual for immediate adaptation to gestational perturbations, with the probability of sequelae in later life (Ganu et al., 2012). Epigenetic modifications to the fetal and placental epigenome accompany these reprogramming events. Mortola (2000) showed that birth weight in humans was less at high altitude than at sea level, once a critical barometric pressure of ~590 mmHg was reached. Moore (2003) also showed that high altitude reduces birth weights, averaging a 100 g fall per 1000 m elevation gain. Several comparisons and considerations suggested that hypoxia was the most important parameter responsible for low birth weight at high altitude, acting independently, not interactively, with other risk factors to reduce birthweight (Jensen and Moore, 1997). Similar observations have been performed in rodents (Nelson and Cons, 1975; Chen et al., 2012, Giussani et al., 2012).

These findings suggest the existence of adaptive changes during prenatal development under hypoxic conditions that could induce long-term consequences on the regulation of appetite and body mass growth. Previously published results from Chen et al. (2012) have shown a higher than normal body weight on the 21 postnatal day in rats born from dams that were subjected to hypobaric hypoxia simulating 5000 meters altitude from gestational day 14 up to delivery. Similarly, Giussani et al. (2012) found normal body weights in 4-month-old rats who were born from rats that were exposed to isobaric hypoxia (13%O_2) from gestational day 6 to 20. Analysis of these findings suggests that the offspring of hypoxic dams maintain the capacity to growth normally during postnatal life under sea level conditions. It is noteworthy that in both studies the pregnant rats were subjected to hypoxia suddenly, without previous acclimation, and that exposure was limited to the final period of pregnancy.

In the present study, we report the body mass growth pattern and the adult body mass in rats that were born from dams previously acclimated to SHA (5400 m) and maintained in the hypoxic milieu during the whole gestation period and nursing. The main purpose of the study was to establish whether gestational and early postnatal altitude hypoxia is able to modify the body mass growth trajectory up to adulthood when the offspring is raised under normoxic conditions, thus creating life-long consequences on body mass development.

Materials and Methods

Hypoxia was induced by placing animals into SHA chambers in which the desired air pressure (380 mmHg, 5450 m) was maintained using vacuum pumps and adjustable inflow valves. Exposure was continuous with short periodic interruptions to replace food and water, clean animal cages, and perform experimental maneuvers when necessary. These actions were conducted at the same time in the control normoxic animals. Experimental subjects were Sprague-Dawley rats. Mature animals of both sexes were divided into two groups, normoxic (NX) and hypoxic (HX). The former group was maintained under ground-level conditions, while the second one was maintained in the SHA chambers during 1 month to permit acclimation to the hypoxic milieu. Mating was then allowed under either normoxic or hypoxic conditions. Offspring was divided into four groups: 1) NX-NX (rats born in normoxia and maintained in normoxia); 2) NX-HX (rats born in normoxia and changed to hypoxia when aged 30 days); 3) HX-HX (rats born in hypoxia and maintained in hypoxia); and 4) HX-NX (rats born in hypoxia and changed to normoxia when aged 30 days).

The experimental period was thus divided into two subperiods, A and B (Fig. 1).The former lasted from conception

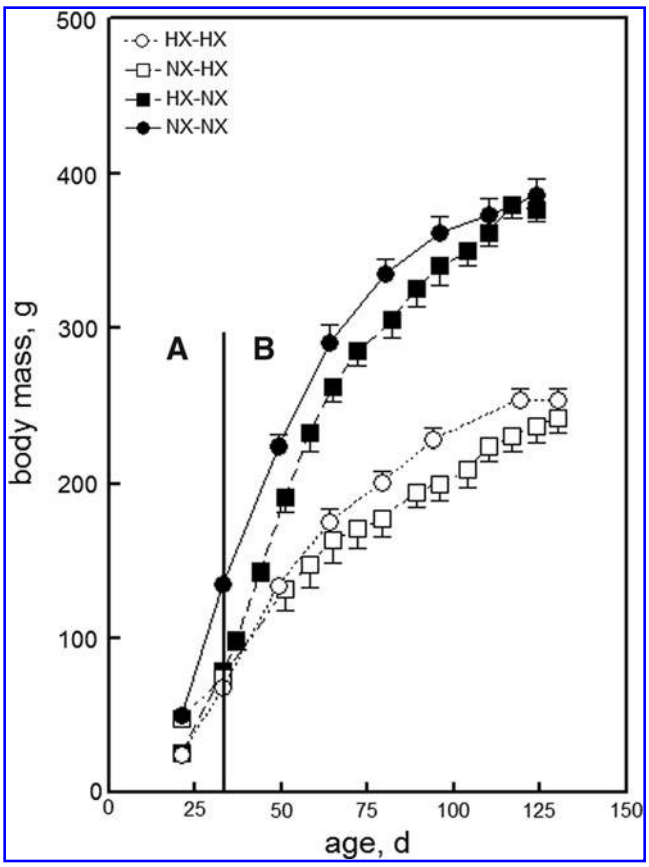

FIG. 1. Body mass as function of age in male rats under various environmental conditions. **(A)** indicates the period that lasted between conception and day 30 of life (day 0 in the graph corresponds to the day of birth). Rats were weaned at day 21. During this period, rats were maintained under either normoxic (NX) or hypoxic (HX, 380 mmHg, 5450 m) conditions. **(B)** indicates the period that lasted between days 30 and 133 of life. During this period, rats were also maintained under either normoxic (NX) or hypoxic conditions. Groups were as follows: 1) HX-HX: *white circles*; Hypoxia during periods A and B; 2) NX-HX: *white squares*; Normoxia during period A and hypoxia during period B; 3) HX-NX: *black squares*; Hypoxia during period A and normoxia during period B; and 4) NX-NX: *black circles*; Hypoxia during periods A and B. Each value represents the mean ± SEM of 6 rats.

Table 1. Body Mass Growth Rate and Final Body Mass in Rats Born and/or Raised under Normoxic or Hypoxic Conditions

Group	Body Mass Growth Rate (g/day)	Final Body Mass (g)
NX-NX	4.86 ± 0.40^{a}	395.14 ± 10.79^{a}
NX-HX	2.76 ± 0.43^{b}	242.24 ± 12.30^{b}
HX-HX	3.05 ± 0.45^{b}	243.36 ± 6.87^{b}
HX-NX	5.75 ± 0.75^{a}	376.40 ± 7.13^{a}

HX-HX, rats born and raised in hypoxia; HX-NX, rats born in hypoxia and raised in normoxia; NX-NX, rats born and raised in normoxia; NX-HX, rats born in normoxia and raised in hypoxia. Data are presented as mean ± SEM. $N = 6$ in each group. Equal letters indicate $p > 0.05$.

to day 30 of postnatal life, while the latter corresponded to the period between day 30 and day 133 of postnatal life. In each group, data from six male animals were analyzed. Male rats were used because their body mass growth rate is higher than that of females, which makes the differences between normoxic and hypoxic animals more marked. However, female rats were simultaneously studied in the present experiment and the results obtained, which are not shown, were qualitatively similar to those seen in males.

Results were summarized as means ± SEM and presented graphically for easier interpretation. Data were analyzed using one-way ANOVA (GraphPad Prism version 4.00 for Windows, GraphPad Software Inc., San Diego, CA, USA). Differences in the means were considered statistically significant when $p < 0.05$.

The experiment was conducted in accordance with the principles outlines in the National Institute of Health Guide for the Care and Management of Laboratory Animals, and approved by the University of Buenos Aires Ethics Committee.

Results and Discussion

Exposure of dams to SHA did not affect the litter size and survival rate at birth significantly. Weight of rats at the time of weaning (21 days) was 49.53 ± 3.28 g in those raised under normoxic conditions (NX) and 25.45 ± 2.12 g in those animals raised at SHA (HX) ($p < 0.0001$), as expected from previously reported studies (Farahany et al., 2008; Moore et al., 2011; Chen et al., 2012; Giussani et al., 2012) that showed that chronic hypoxia slows fetal and perinatal growth. Whether these effects are due to different factors, such as neuronal development, endocrine state, and behavior patterns (Peters, 1988; Champagne et al., 2003) or merely represent the known inhibitory effect of hypoxia on body mass, as occurs during later life periods, remains as an open question.

As shown in Figure 1, the body mass increased with time in all groups under both normoxic and hypoxic conditions. The body mass growth rates between days 33 and 65, a period of postnatal life in which body mass increases roughly linearly, are shown in Table 1. Two observations are worth-noting: 1) Rats living under hypoxic air during period A of the experiment showed a BMGR when exposed to normoxic air during period B that was not significantly different from those living under normoxic conditions during period A (NX-NX vs. HX-NX). The 18% increment in the BMGR observed in the HX-NX group in relation to the NX-NX group allowed catch-up growth in the former to approach the normal growth curve after cessation of the negative effects of hypoxia on growth during period A; and 2) Rats living under normoxic air during period A showed a BMGR when exposed to hypoxic air during period B that was not significantly different from those living under hypoxic conditions during period A (NX-HX vs. HX-HX). Analysis of these data indicates that SHA conditions negatively affected body mass growth during postnatal life irrespective of whether the animals were gestated, born, and lived during pre- early postnatal life under either normobaric of hypobaric ambient conditions. However, they did not affect growth under normobaric conditions.

Body mass of animals was measured at the end of subperiod B of the experimental period, when animals were aged 133 days (young adults). Values are shown in Table 1. Analysis of these data indicates that exposure to SHA conditions between the 30 and the 133 days of postnatal life reduced the body mass of animals in approximately 37% when compared to those maintained under normal ambient conditions. The effect was not related to the previous exposure to hypoxia during gestation and 30-day early postnatal life.

The results of this study suggest that hypobaric hypoxia during prenatal and early postnatal (30 days) development does not alter the regulation of body mass growth in rats when compared to that seen under sea-level conditions. This conclusion agrees with those previously commented from Chen et al. (2012) and Giussani et al. (2012), although it is clear that hypobaric hypoxia throughout pregnancy (present study) is different from hypobaric hypoxia along gestational days 14–21 (Chen et al., 2012) and also from isobaric hypoxia along gestational days 6–20 (Giussani et al, 2012). Therefore, it is concluded that no deleterious consequences of gestational and perinatal exposure to hypoxia on body mass growth under normobaric conditions are thus evident, but that nonetheless hypoxia, when present postnatally, reduces body growth.

Acknowledgments

This investigation was supported by Research Grants from the University of Buenos Aires (UBACYT 20020100100067 and 20020100100389). CEB and RMA are Career Investigators from National Research Council (CONICET).

Author Disclosure Statement

No conflicting financial interests exist.

References

Alippi RM, Barceló AC, Río ME, and Bozzini CE. (1983). Growth retardation in the early developing rat exposed to continuous hypobaric hypoxia. Acta Physiol Latinoam 33: 1–5.

Baker DJP. (1994). *Mothers, Babies and Disease in Later Life.* B.M.J. Publishing Group, London, UK.

Bozzini CE, Boyer PM, Friedman SM, Lezón CE, Norese MF, and Alippi RM. (1998). Hypophagia-induced hypometabolism during experimental anaemia in rats. Comp Haemat Internat 8:197–201.

Bozzini C, Champin G, Alippi RM, and Bozzini CE. (2013). Static biomechanics in bone from growing rats exposed chronically to simulated high altitudes. High Alt Med Biol 14:367–374.

Champagne FA, Francis DD, Mar A, and Meaney MJ. (2003). Variation in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav 79:359–371.

Chen F, Du S, Bian J, You ZB, and Wu Y. (2012).Chronic hypoxia exposure during pregnancy is associated with a decreased active nursing in mother and an abnormal birth weight and postnatal growth in offspring of rats. Horm Behav 61:504–511.

Delaquierre-Richardson L, Forbes S, and Valdivia E. (1971). Effect of simulated high altitude on the growth rate of albino guinea pigs. J Appl Physiol 20:1022–1025.

Elia R, Elgoyhen AB, Bugallo G, Rio ME, and Bozzini CE. (1985). Effect of reduced atmospheric pressure on body weight, food intake and body composition of growing rats. Acta Physiol Latinoam 35:311–318.

Farahany R, Kanaan A, Gavrialov O, Brunnert S, Douglas RM, Morcillo P, and Haddad GG. (2008). Differential effect of chronic intermittent and chronic constatnt hypoxia on postnatal growth and development. Pediatr Pulmonol 43:20–28.

Ganu RS, Harris RA, Collins K, and Aagaard KM. (2012). Early origins of adult disease: Approaches for investigating the programmable epigenome in humans, nonhuman primates, and rodents. ILAR J 53:306–321.

Giussani DA, Camm EJ, Niu Y, Richter HG, Blanco CE,-Gottschalk R, Zachary Blake E, Horder KA, Thakor AS, Hansell JA, Kane AD, Wooding FB, Cross CM, and Herrera MA. (2012). Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. PLoS ONE 7:e31017

Gluckman PD, and Hanson MA. (2007). Developmental plasticity and human disease: research directions. J Intern Med 261:461–471.

Godfrey KM, Lillycrop KA, Butdge GC. Gluckman PD, and Hanson MA. (2007). Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. Pediatr Res 61:5R–10R.

Jensen GM, and Moore LG. (1997). The effect of high altitude and other risk factors on birthweight: Independent or interactive effects? Am J Public Health 87:1003–1007.

Monge C, and León-Velarde F. (1991). Physiological adaptation to high altitude: Oxygen transport in mammals and birds. Physiol Rev 71:1135–1172.

Moore LG. (2003). Fetal growth restriction and maternal oxygen transport during high altitude pregnancy. High Alt Med Biol 4:141–156.

Morel OE, Aubert R, Richalet JP, and Chapelot D. (2005). Simulated high altitude decreases protein intake and lean body mass in rats. Physiol Behav 86:145–153.

Mortola JP. (1993). Hypoxic metabolism in mammals. News Physiol Sci 8:79–82.

Mortola JP, Frapell PB, Aguero L, and Amstrong K. (2000). Birth weight at altitude: A study in Peruvian communities. J Pediatr 136:324–329.

Norese MF, Lezón CE, Alippi RM, Martínez MP, Conti MI, and Bozzini CE. (2002). Failure of polycythemia-induced increase in arterial oxygen content to suppress the anorexic effect of similated high altitude in the adult rat. High Alt Med Biol 3:49–57.

Peters DA. (1988). Both prenatal and postnatal effects of maternal stress on offspring behavior and central 5-hydroxytryptamine receptors in the rat. Pharmacol Biochem Behav 30:669–673.

Schwartz JE, Kovach A, Meyer J, McConnell C, and Iwamoto HS. (1998). Brief, intermittent hypoxia restricts fetal growth in Sprague-Dawley rats. Biol Neonate 73:313–319.

Schnakenberg MW, Krabill LE, and Weisser PC. (1971). The anorexic effect of high altitude on weight gain, nitrogen retention and body composition of rats. J Nutr 101:787–796.

Timiras PS, Krum AA, and Pace N. (1957). Body and organ weights of rats during acclimatization to an altitude of 12470 feet. Am J Physiol 191:598–604.

Westerterp-Plantenga MS, Westerterp KR, Rubbens N, Verwegen CRT, Richalet JP, and Gardette B. (1999). Appetite at high altitude (Operation Everest III (Comex-'97): A simulated ascent of Mount Everest. J Appl Physiol 87:391–399.

Address correspondence to:
Dr. Carlos E. Bozzini
Department of Physiology
Faculty of Odontology
University of Buenos Aires
Marcelo T. de Alvear 2142
Buenos Aires 1122
Argentina

E-mail: cebozi@fisio.odon.uba.ar

Received March 6, 2014;
accepted in final form May 9, 2014.