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The Effect of Hypoxia on Plasma Leptin and Insulin in Newborn and Juvenile Rats

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Hypoxia leads to a decrease in food intake and attenuated weight gain in rats. The purpose of this study was to measure plasma leptin and insulin in young rats exposed to hypoxia for 7 d as compared to a normoxic control group of the same age. One group was exposed from birth to 7 d of age; the other was exposed from 28 to 35 d of age (weaned at 21 d of age). As expected, body weight was significantly lower in rats of either age exposed to hypoxia for 7 d. Plasma leptin was significantly lower in hypoxic (2.0 ± 0.2 ng/mL; *n* = 41) compared with normoxic (2.6 ± 0.3 ng/mL; *n* = 30) 7-d-old rats. Plasma leptin was also significantly lower in hypoxic (1.1 ± 0.1 ng/mL; *n* = 20) as compared to normoxic (1.5 ± 0.1 ng/mL; *n* = 20) 35-d-old rats. Seven-day-old rats exposed to hypoxia demonstrated significant increases in plasma glucose and insulin whereas 35-d-old rats exhibited a decrease in both variables. We conclude that exposure to hypoxia for 7 d leads to a decrease in body weight and plasma leptin in infant and juvenile rats. The decrease in leptin may be an attempt to reverse hypoxia-induced anorexia.

Key Words: Hypoxia; leptin; insulin; neonate; rat; body weight.

Introduction

Loss of appetite and weight loss is a common problem in adults exposed to chronic hypoxia *(1,2)*. Hypoxia in the newborn through the adult rodent also leads to decreased food intake, decreases in body fat, and attenuated weight gain *(3–7)*. Hypoxia-induced weight loss is thought to be primarily a result of anorexia rather than a change in oxygen consumption *(2,7)*.

Although the control of food intake is an extremely complex process, it has been hypothesized that leptin, the monomeric 16-kDa protein product of the *ob* gene, is involved in a negative feedback loop control system such that an increase in leptin leads to satiety. A current theory is that leptin produced from adipocytes signals the satiety centers in the hypothalamus perhaps via hypothalamic neuropeptide Y *(8,9)*. It has also been hypothesized that leptin is an important component in the control of metabolism in early life—from fetal and neonatal through adolescent ages *(10)*.

To our knowledge, the effect of chronic hypoxia on plasma leptin has not been evaluated in neonatal, infant, or adolescent rats. Using a recently developed model of neonatal hypoxia *(11,12)*, we examined the hypothesis that body weight and leptin levels would be lower in newborn and juvenile rats exposed to hypoxia for 7 d. We also hypothesized that changes in plasma glucose and insulin concentration would correlate with plasma leptin as has been shown for other models of weight loss *(9,10)*. Rats were exposed to hypoxia for 7 d either from birth to 7 d of age or from 28 to 35 d of age (weaned at 21 d).

Results

Seven days of hypoxia led to a significantly lower body weight in either age group of rats. Body weight in 7-d-old hypoxic rats (9.8 ± 0.2 g) was 37% lower than in 7-d-old normoxic rats (15.7 ± 0.4 g). Body weight in 35-d-old rats exposed to hypoxia for 7 d (101 ± 3 g) was 10% lower than 35-d-old normoxic rats (112 ± 3 g).

Figure 1 shows plasma leptin, insulin, and glucose levels in rats exposed to normoxia vs hypoxia from birth to 7 d of age and from 28 to 35 d of age. Under normoxic conditions, plasma leptin was significantly higher in 7-d-old rats compared with 35-d-old rats. Exposure to hypoxia for 7 d led to a significant decrease in plasma leptin levels in rats of both ages. Plasma glucose was significantly higher in hypoxic 7-d-old rats compared with normoxic 7-d-old rats. There was a small but significant decrease in plasma glucose in 35-d-old hypoxic vs normoxic rats. Plasma insulin reflected the changes in plasma glucose with insulin being significantly increased in 7-d-old hypoxic rats and significantly decreased in 35-d-old hypoxic rats. Despite similar plasma glucose levels, 35-d-old normoxic rats had significantly higher plasma insulin compared with 7-d-old normoxic rats.

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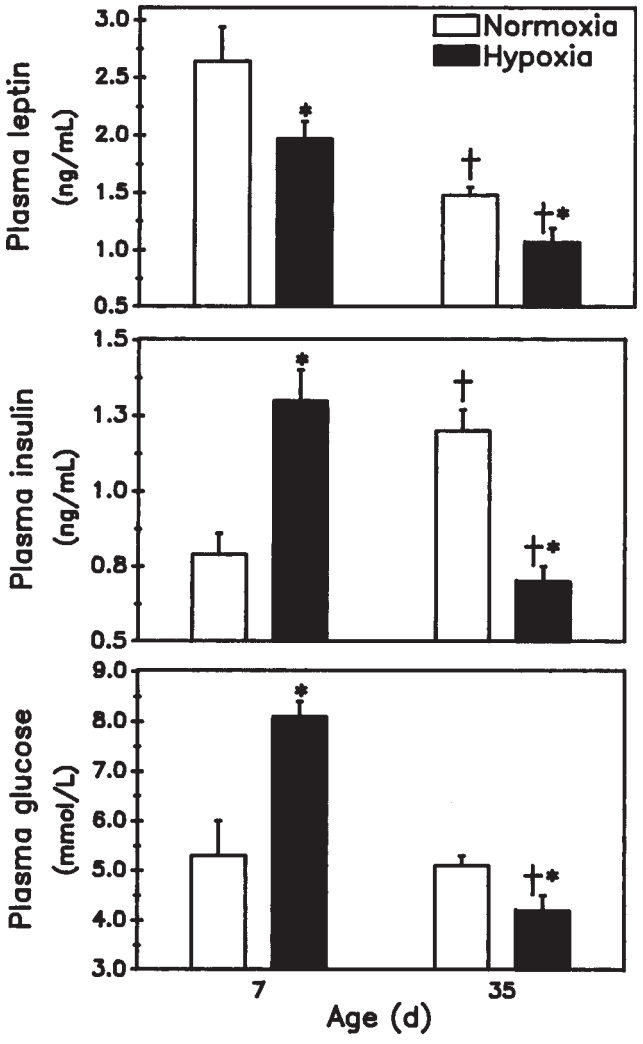

Fig. 1. Plasma leptin, insulin, and glucose levels of rats exposed to hypoxia for 7 d either from birth to 7 d of age (n = 30–40 per mean) or from 28 to 35 d of age (weaned at 21 d of age; n = 20–41 per mean). *, hypoxic significantly different from normoxic rats (p <0.05); †, 35-d-old significantly different from 7-d-old.

Discussion

This study demonstrated, for the first time, to our knowledge, that exposure to hypoxia results in a decrease in plasma leptin levels in the infant and juvenile rat. This was associated with a decrease in body weight. Also of interest was that leptin levels in normoxic control rats decreased from 7 to 35 d of age. Whereas the hypoxia-induced decrease in leptin was paralleled by a decrease in plasma insulin and glucose in the 35-d-old rats, there was a marked divergence of leptin and insulin/glucose in the 7-d-old rats with hypoxia leading to significant increases in both plasma glucose and insulin.

The decrease in leptin likely results from a decrease in body weight and adipocity during hypoxia *(3–7)* although we cannot rule out a small direct effect of hypoxia on the expression of the *ob* gene. The anorexia associated with hypoxia is a significant problem both in normal individuals at altitude and in specific cardiopulmonary disorders. This weight loss is considered to be primarily owing to a decrease in food intake *(7)* since enforced maintenance of food intake prevents weight loss *(2)*. However, maintenance of adequate caloric intake is difficult in most severely hypoxic individuals *(1–7)*. This may be particularly problematic in newborn and infant humans who require supplementation of caloric intake *(13)*.

Note also that leptin levels in control (normoxic) rats decreased between 7 and 35 d of age. A previous study found similar changes in leptin between 10- and 35-d-old rats and conjectured that the relatively low leptin levels in weanling rats may be owing to loss of leptin intake from dam's milk *(14)*.

Previous studies of plasma glucose and insulin during hypoxia have led to conflicting results, and this area of study is by no means resolved *(15)*. Several articles have demonstrated that adult rats and humans have small increases in plasma insulin without a significant change in plasma glucose *(3,16)*. Nevertheless, the changes at 35 d of age we observed in the current study were as expected since a decrease in food intake leading to weight loss is known to decrease leptin, insulin, and glucose *(9,10)*. However, exposure to hypoxia from birth in the 7-d-old rats led to significant hyperglycemia and hyperinsulinemia. This is likely a reflection of the marked increase in counterregulatory hormones (e.g., corticosterone) that occurs in the 7-d-old compared with older rats *(11,17)*. Therefore, in the suckling rat, hypoxia-induced anorexia led to weight loss, decreased adipocity, and decreased leptin and increased counterregulatory hormones led to an increase in plasma glucose and insulin. Despite similar plasma glucose levels, insulin was higher in 35-d-old control (normoxic) rats compared with 7-d-old control rats. This phenomenon has been suggested previously and may be related to changes in thyroid hormone secretion *(18)*.

The hypoxia-induced decrease in leptin observed in our study can be considered a normal homeostatic adaptation to attempt to increase food intake by decreasing satiety. Despite the decrease in leptin, rats still failed to maintain their body weight, indicating the potent anorectic effect of hypoxia. It remains to be determined whether pharmacological manipulation of the leptin system can ameliorate the anorectic effects of chronic hypoxia.

Materials and Methods

Animal Treatment

All experimentation was approved by the Institutional Animal Care and Use Committees of the Medical College of Wisconsin and St. Luke's/Sinai Samaritan Medical Center. Timed pregnant Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN, N = 12) were obtained at 14 d of gestation and maintained on a standard sodium diet (Rich-

mond Standard #5001, Brentwood, MO) and water ad libitum in a controlled environment (lights on from 6:00 AM to 6:00 PM). Parturition usually occurred on the afternoon of gestational d 21, during which rats were kept under observation.

Hypoxia from 0 to 7 d of Age

As soon as a litter was completely delivered, the dam and her pups (8–10/litter, male and female) were immediately moved to an environment chamber exposed to normobaric normoxia (21% O_2; room air) or hypoxia (12% O_2) as described in detail previously *(11,17,19)*. We have previously shown that this exposure leads to arterial PO_2 levels in adults of about 50–55 torr with sustained hypocapnia and alkalosis *(17,19)*. Lactating dams were maintained with their litters for 7 d in a normoxic or hypoxic environment *(11,12)*. Chambers were briefly opened on d 4 to clean the cages. At 8:00 AM of d 7, dams were quickly removed from the chambers. Then rat pups were quickly weighed and decapitated, and trunk blood was collected for the measurement of plasma leptin, glucose, and insulin.

Hypoxia from 28 to 35 d of Age

Male and female rats from randomly assigned litters were weaned at 21 d of age. At 28 d of age, they were placed in chambers and exposed to hypoxia for 7 d, and trunk blood was collected as described previously.

Measurements and Statistical Analyses

Body weight in 7-d-old rats was measured using an Ohaus LS200 electronic balance (Florham Park, NJ). Body weight in 35-d-old rats was measured using an Ohaus Dial-o-Gram balance (Florham Park, NJ). Plasma glucose was measured spectrophotometrically using reagents purchased from Sigma (St. Louis, MO). Plasma leptin and insulin were measured in duplicate by radioimmunoassays using commercially available kits (Linco, St. Charles, MO). Intraassay and interassay coefficients of variation (CVs) of the leptin assay were 3–6%; the lowest standard was 0.5 ng/mL. The interassay CV of the insulin assay was 6% (all the samples were measured on the same day); the lowest standard was 0.1 ng/mL. Plasma data were analyzed by two-way analysis of variance (normoxia/hypoxia vs age) followed by Duncan's multiple range test. $p<0.05$ was considered significant. Data are presented as mean ± SE.

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References

1. Cerretelli, P. and Hoppeler H. (1996). In: *Handbook of Physiology. Section 4. Environmental Physiology, volume II*. Fregly, M. J. and Blatteis, C. M. (eds.). Oxford University: New York.
2. Butterfield, G. E., Gates, J., Fleming, G., Brooks, G. A., Sutton, J. R., and Reeves, J. T. (1992). *J. Appl. Physiol.* **72,** 1741–1748.
3. Singh, S. B., Sharma, A., Sharma, K. N., and Selvamurthy, W. J. (1996). *J. Appl. Physiol.* **80,** 1133–1137.
4. Moromisato, D. Y., Moromisato, M. Y., Zanconato, S., Roberts, C. T., Brosel, J. A., and Cooper, D. M. (1996). *Crit. Care Med.* **24,** 919–924.
5. Gloster, J., Hasleton, P. S., Harris, P., and Heath, D. (1974). *Environ. Physiol. Biochem.* **4,** 251–258.
6. Fushiki, T., Kano, T., Ito, K., Hirofuji, D., Inoue, K., Moritani, T., and Sugimoto, E. (1992). *Can. J. Physiol. Pharmacol.* **70,** 1522–1524.
7. Elia, A., Elgoyhen, A. B., Bugallo, G., Rio, M. E., and Bozzini, C. E. (1985). *Acta Physiol. Pharmacol. Latinoam.* **35,** 311–318.
8. Buchanan, C., Mahesh, V., Zamorano, P., and Brann, D. (1998). *Trends Endocrinol. Metab.* **9,** 146–150.
9. Fruhbeck, G., Jebb, S. A., and Prentice, A. M. (1998). *Clin. Physiol.* **18,** 399–419.
10. Kiess, W., Siebler, T., Englaro, P., Kratzsch, J., Deutscher, J., Meyer, K., Gallaher, B., and Blum, W. F. (1998). *J. Pediatr. Endocrinol. Metab.* **11,** 483–496.
11. Raff, H., Jankowski, B. M., Bruder, E. D. Engeland, W. C., and Oaks, M. K. (1999). *Endocrinology* **140,** 3147–3153.
12. Thomas, T. and Marshall, J. M. (1995). *J. Physiol.* **487,** 513–525.
13. Harris, R. J. (1974). *J. Pediatr.* **84,** 578–584.
14. Trottier, G., Koski, K. G., Brun, T., Toufexia, D. J., Richard, D., and Walker, C.–D. (1998). *Endocrinology* **139,** 3704–3711.
15. Raff, H. (1996). In: *Handbook of Physiology Section 4: Environmental Physiology*. Fregly, M. J. and Blatteis, C. M. (eds.). Oxford University Press: New York.
16. Sawhney, R. C., Malhotra, A. S., Singh, T., Rai, R. M., and Sinha, K. C. (1986). *Int. J. Biometeor.* **30,** 231–238.
17. Raff, H., Sandri, R. B., and Segerson, T. P. (1986). *Am. J. Physiol.* **250,** R240–R244.
18. Ramos, S., Goya, L., Alvarez, C., and Pascual-Leone, A. M. (1998). *Endocrinology* **139,** 4782–4792.
19. Raff, H. and Chadwick, C. J. (1986). *Clin. Exper. Pharmacol. Physiol.* **13,** 827–830.