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Effect of chronic hypoxia on leptin, insulin, adiponectin, and ghrelin

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Abstract

The endocrine system plays an important role in the adaptation to hypoxia. The aim of this study was to assess the effect of chronic hypoxia on insulin, adiponectin, leptin, and ghrelin levels in a neonatal animal model. Sprague-Dawley rats were placed in a normobaric hypoxic environment at birth. Controls remained in room air. Rats were killed at 2 and 8 weeks of life. Insulin, adiponectin, leptin, and ghrelin were measured. At 2 weeks of life, there was no significant difference in insulin, adiponectin, and leptin levels between the hypoxic and control rats. The only statistically significant difference was found in ghrelin levels, which were lower in the hypoxic group (3.19 ± 3.35 vs 24.52 ± 5.09 pg/mL; $P < .05$). At 8 weeks of life, insulin was significantly higher in the hypoxic group (0.72 ± 0.14 vs 0.44 ± 0.26 ng/mL; $P < .05$) and adiponectin was significantly lower (1257.5 ± 789.5 vs 7817.3 ± 8453.7 ng/mL; $P < .05$). Leptin and ghrelin did not show significant difference in this age group, but leptin level per body weight was higher in the hypoxic group. Finally, we conclude that 2 weeks of continuous neonatal hypoxic exposure leads to a decrease in plasma ghrelin only with no significant change in insulin, adiponectin, and leptin and that 8 weeks of hypoxia leads to a decrease in adiponectin with an increase in insulin despite a significant decrease in weight.
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Keywords: Hypoxia; Adiponectin; Insulin; Leptin; Ghrelin

1. Introduction

The endocrine system has an important role in the adaptation to hypoxia and stress and is reciprocally affected by these situations [1]. The effect of hypoxia on the endocrine system was studied in men at high altitude [2-5] and in animals exposed to hypoxia [6-10]. This effect is not only limited to hormonal secretions, it can also extend to affect the morphology and histology of the endocrine glands [6,7,11]. Hypoxic stress is well known to decrease appetite and weight gain in growing rats and to induce weight loss in humans at high altitude [12,13]. It also increases the expression of a variety of genes with products that act in synergy to facilitate the supply of metabolic energy [14]. In this regard, hypoxia induces leptin gene promoter leading to an increase in its level [15-17] and increases insulin messenger RNA [18]. Insulin and hypoxia act synergistically to induce leptin transcription [19]. In addition, hypoxia affects adiponectin but has no effect on ghrelin levels. Wolk et al [20] showed elevated adiponectin levels in men with obstructive sleep apnea, and Raff [12] showed that hypoxia had no effect on ghrelin.

Because of the emerging concept of the adipose tissue as an endocrine gland [21] and the effect of hypoxia on metabolic energy and weight [14,22], we tried to look at the effect of hypoxia on insulin, leptin, adiponectin, and ghrelin levels in a well-established rat model of chronic normobaric hypoxia.

2. Study design and methods

All animals received care in accordance with approved institutional animal care guidelines and according to the

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Guide for Care and Use of Laboratory Animals of the National Academy of Science and the Principles of Laboratory Animal Care of the National Society of Medical Research.

As previously described, an animal model mimicking cyanotic heart disease was studied [23,24]. Animal cages were placed in temperature-controlled rooms (22°C) with constant 12-hour light/dark cycle. Standard laboratory rat chow and tap water were provided ad libitum. Soda lime was placed inside the chamber to absorb carbon dioxide, and carbon dioxide levels were kept less than 1% to 2%. Nitrogen and oxygen tanks with gauge flow were attached into a specially built airtight Plexiglas (Trident Plastics, Ivyland, PA) chamber at standard cage dimensions. Oxygen levels were monitored continuously by an oxymeter and maintained at 10% in the Plexiglas chamber (atmospheric oxygen was about 50% of normal levels). Air within the chamber was recycled after being passed through an anhydrous calcium sulfate and silica gel mixture to remove ammonia and moisture.

Sprague-Dawley rats from our established breeding colony were used in the study. Female pregnant rats were followed until delivery occurred and pups were randomly selected into 2 groups: the hypoxic group (H) and the control (C) group. In each set, rats were assigned a specific time for killing, that is, 2 and 8 weeks. Rats selected to be in a normobaric hypoxic environment were transferred to the chamber within a few minutes from birth, whereas the control group remained in room air. Hypoxic animals were maintained in the chamber for the remaining of the experimental period. The dam stayed with its rat pups in the Plexiglas chamber and tolerated the hypoxic environment. Control rats were reared in room air and were divided into 2 groups: group 2C (n = 13) consisted of 2-week-old rats and group 8C (n = 11), of 8-week-old rats. On the other hand, the hypoxemic groups were group 2H (n = 12; 2-week-old rats) and group 8H (n = 7; 8-week-old rats). Pups were weaned at 3 to 4 weeks of age, at that time they received the same type of chow fed as the dam; in addition, they continued receiving lactation, occasionally.

Rat pups aged 2 and 8 weeks were killed under deep pentobarbital anesthesia (50 mg/kg IP). Hematocrit levels were measured and animals were weighed. Killing was performed at the same time each morning to eliminate the influence of possible diurnal variations on endocrinologic measurements, and rats were kept fasting to eliminate the influence of food on the measurements.

2.1. Endocrine tests

In control and hypoxic rats, leptin, ghrelin, adiponectin, and insulin levels were measured at 2 and 8 weeks of life.

Plasma leptin was analyzed using Linco's Rat Leptin Radioimmunoassay (RIA) Kit, plasma ghrelin using Linco's Ghrelin (Active) RIA Kit, plasma adiponectin using Linco's Mouse Adiponectin RIA Kit, and plasma insulin using the Sensitive Rat Insulin RIA Kit. All these plasma levels were determined according to the manufacturer's instructions (Linco Research, St Charles, MO).

2.2. Statistical analysis

Hypoxic and control groups of the same age were compared using an unpaired *t* test; and when variables did not have a normal distribution, the nonparametric Mann-Whitney test was used. A *P* value of less than .05 was considered statistically significant. All data were expressed as mean ± SD.

3. Results

3.1. Body weight and hematocrit levels

Weights of rats were significantly lower in the hypoxic group compared to the controls with a *P* value of less than .001 (weight at 2 weeks: 29.2 ± 3.1 [C] vs 18.1 ± 2.2 [H], $P < .001$; weight at 8 weeks: 217.5 ± 34.6 [C] vs 128.2 ± 22.5 [H], $P < .001$). Significant polycythemia developed in the hypoxic rats compared to controls, respectively, with $P < .001$. This polycythemic response is an expected response to chronic hypoxia and validates our hypoxic model (hematocrit level at 2 weeks: 33.2 ± 2.1 [C] vs 43.2 ± 2.6 [H], $P < .001$; hematocrit level at 8 weeks: 41.4 ± 3.8 [C] vs 54.6 ± 2.6 [H], $P < .001$).

3.2. Endocrine tests

At 2 weeks of life, insulin, ghrelin, adiponectin, and leptin levels were compared (Table 1); the only statistically significant difference was found in ghrelin levels where the hypoxic group had lower levels (3.19 ± 3.35 pg/mL for the 2H group vs 24.52 ± 5.09 pg/mL for the 2C group; $P < .05$). Because of the difference in weight between the 2 groups, at 2 weeks, when we expressed the data per body weight, there was no difference between the groups in the levels of insulin/body weight, adiponectin/body weight, and leptin/body weight.

At 8 weeks, the hypoxic group had higher levels of insulin (0.72 ± 0.14 ng/mL [8H] vs 0.44 ± 0.26 ng/mL [8C]; $P < .05$) and lower levels of adiponectin (1257.5 ± 789.5 ng/mL [8H] vs 7817.3 ± 8453.7 ng/mL [8C]; $P < .05$), both statistically significant. Leptin levels tended to be higher in the hypoxic

Table 1
Effect of hypoxia on weight, hematocrit, insulin, ghrelin, adiponectin, and leptin levels at 2 weeks of neonatal age in Sprague-Dawley rats

	Control	Hypoxic	*P*
Weight (g)	29.2 ± 3.1	18.1 ± 2.2	<.001
Hematocrit	33.2 ± 2.1	43.2 ± 2.6	<.001
Insulin (ng/mL)	0.35 ± 0.17	0.25 ± 0.25	NS
Ghrelin (pg/mL)	24.5 ± 5.09	3.19 ± 3.35	<.05
Adiponectin (ng/mL)	1710.9 ± 1423.4	1930.9 ± 3105.0	NS
Leptin (ng/mL)	0.23 ± 0.22	0.19 ± 0.16	NS

Data are reported as means ± SD. NS indicates not significant.

Table 2
Effect of hypoxia on weight, hematocrit, insulin, ghrelin, adiponectin, and leptin levels at 8 weeks of neonatal age in Sprague-Dawley rats

	Control	Hypoxic	*P*
Weight (g)	217.5 ± 34.6	128.2 ± 22.5	<.001
Hematocrit	41.4 ± 3.8	54.6 ± 2.6	<.001
Insulin (ng/mL)	0.44 ± 0.26	0.72 ± 0.14	<.05
Ghrelin (pg/mL)	18.69 ± 5.80	10.48 ± 10.85	NS
Adiponectin (ng/mL)	7817.3 ± 8453.7	1257.5 ± 489.5	<.05
Leptin (ng/mL)	0.76 ± 0.37	0.89 ± 0.43	NS

Data are reported as means ± SD.

group without reaching significant difference (0.89 ± 0.42 ng/mL [8H] vs 0.76 ± 0.36 ng/mL [8C]). Ghrelin levels tended to be lower in the hypoxic group, also without reaching significant difference (10.48 ± 10.85 pg/mL [8H] vs 18.69 ± 5.79 pg/mL [8C]) (Table 2).

Because of the difference in weight between the 2 groups, we expressed leptin levels per body weight in the 8-week groups. Leptin/weight was higher in the hypoxic group: 6.95×10^{-3} ng mL^{-1} kg^{-1} (8H) vs 3.49×10^{-3} ng mL^{-1} kg^{-1} (8C) ($P < .05$).

4. Discussion

Reports by Benette et al have shown that leptin may have independent effect on erythropoiesis and the increased leptin levels in patients with renal failure may be a response to anemia [25,26]. Also, Bornstein et al [27] demonstrated that plasma leptin levels are increased in survivors of acute sepsis, which means that increased leptin levels is a predictor for survival in septic shock patients. Based on these reports, tissue hypoxia could well be a mechanism for stimulating leptin secretion.

In our animal study, hypoxic rats were significantly more polycythemic than the controls and had more weight loss, which is expected and confirms that our hypoxic rats were truly hypoxemic. Hypoxic stress usually induces weight loss during high mountain expeditions by decreased energy expenditure, increased metabolic rate [13], and loss of appetite [14]. Weight loss was evident in the hypoxic groups at 2 and 8 weeks of life. It coincided with a significant decrease in adiponectin and increase in insulin after 8 weeks and with a significant decrease in ghrelin after 2 weeks. Adiponectin is an adipocytokine with an important role in energy homeostasis and insulin sensitivity and is inversely correlated to the degree of adiposity. Raff et al [28] previously showed no change in adiposity during neonatal hypoxia. The adiponectin level per body weight was still lower in the hypoxic group than in the control group, suggesting that hypoxia may have a direct effect on this hormone independent of weight. Hence, adiponectin decrease in the 8-week hypoxic rats suggests that multiple factors interact to decrease adiponectin levels during hypoxia independent of the adiposity. This decrease in adiponectin was not described previously. Wolk et al [20], on the contrary, showed high levels of adiponectin in patients with obstructive sleep apnea, which is a model of intermittent hypoxia. Ghrelin levels were lower in the hypoxic group at 2 weeks, suggesting that this may be contributing to the initial weight loss. However, at 8 weeks, hypoxia had no effect on ghrelin. Previously, Raff [12] demonstrated that hypoxia had no effect on plasma ghrelin after exposing rats to hypoxia for 7 days as neonates (birth-7 days of age), weanlings (28-35 days of age), and juveniles (49-56 days of age). No previous study addressed the effect of permanent neonatal hypoxia since birth on adiponectin and ghrelin levels.

Insulin levels are expected to drop with weight loss; however, in the hypoxic group and despite weight loss, insulin levels were higher than in the control group. This implies that hypoxia has a direct effect on insulin independent of weight changes. Hypoxia was previously shown to increase insulin messenger RNA [18]. And in turn, insulin induces leptin production in human placenta and acts synergistically with hypoxia to stimulate leptin gene expression [19]. Baum and Porte [29,30] were the only group to report the inhibition of insulin release during hypoxia when studying hypoxic puppies. Their results are in contradiction with our study and with most of previously published studies [18,19,23,28].

Leptin tended to be higher after 8 weeks of long-term exposure to hypoxia, but a significant difference was not reached most probably due to the small sample size. Previous studies showed that leptin levels increase during hypoxia [16,17,31,32]. The effect of hypoxia on leptin was demonstrated at multiple levels: Grosfeld et al [16] reported an increase in leptin gene expression and Ambrosini et al [15] showed that the leptin gene is transcriptionally activated, placenta-derived leptin is increasingly secreted with placental hypoxia, and leptin expression in cells cultured under hypoxic conditions was higher than under standard conditions as demonstrated by Mise et al [31]. Weight loss is expected to decrease leptin level; however, despite weight loss in the hypoxic group, leptin levels tended to be higher. A higher leptin per body weight content was observed in our hypoxic group at 8 weeks, suggesting that, most probably, the origin of the high leptin is not only from the adipocytes. Although clinically leptin serves as a useful marker of adiposity, reflecting the total lipid content in humans [33], Yasumasu et al [34] showed that hypoxia does not stimulate leptin secretion from adipocytes; leptin secretion during this condition comes from non-adipocytes.

Finally, we conclude that 2 weeks of continuous neonatal hypoxic exposure leads to a decrease in plasma ghrelin and to an increase in insulin and leptin (relative to the body weight) and that 8 weeks of hypoxia leads to a decrease in adiponectin and an increase in insulin despite a significant decrease in weight. No significant change in leptin and ghrelin was noted at 8 weeks; however, leptin per body weight was higher in the hypoxic group.

References

[1] Health D, Williams DR. Endocrines. Man at high altitude. 2nd ed. Edinburgh London: Churchill Livingstone; 1981. p. 250-4.

[2] Braun B, Rock PB, Zamudio S, et al. Women at altitude: short-term exposure to hypoxia and/or α1adrenergic blockade reduces insulin sensitivity. J Appl Physiol 2001;91:623-31.

[3] Gallon V. Some effects of altitude on thyroid function. Endocrinology 1972;91:1393-403.

[4] Larsen JJ, Hansen JM, Olsen NV, et al. The effect of altitude hypoxia on glucosa homeostasis in men. J Physiol 1997;504:241-9.

[5] Mordes JP, Blume FD, Boyer S, et al. High altitude pituitary thyroid dysfunction on Mount Everest. N Engl J Med 1983;308:1135-8.

[6] Garvey D, Akana S, Weisman A, et al. Alterations in adrenal growth and corticosteroid content in foetal and neonatal rats developing at high altitude. J Endocrinol 1979;80:333-42.

[7] Gosney JR. Morphological changes in the pituitary and thyroid of the rat in hypobaric hypoxia. J Endocrinol 1986;109:119-24.

[8] Hermans RH, Longo LD. Altered catecholaminergic behavioral and hormonal responses in rats following early postnatal hypoxia. Phys Behav 1994;55:469-75.

[9] Jacobs R, Robinson JS, Owens JA, et al. The effect of prolonged hypobaric hypoxia on growth of fetal sheep. J Dev Physiol 1988;10: 97-112.

[10] Zayour D, Azar ST, Azar N, et al. Endocrine changes in a rat model of chronic hypoxia mimicking cyanotic heart disease. Endocr Res 2003; 29:91-200.

[11] Gosney J, Health D, Williams D, et al. Morphological changes in the pituitary-adrenocortical axis in natives of La Paz. Int J Biometeorl 1991;35:1-5.

[12] Raff H. Total and active ghrelin in developing rats during hypoxia. Endocrine 2003;21:159-61.

[13] Tschop M, Morrison KM. Weight loss at high altitude. Adv Exp Med Biol 2001;502:237-47.

[14] Yasumasu T, Takahara A, Nakashima Y. Reply to: leptin is a hypoxia inducible gene. Obes Res 2002;10:857-8.

[15] Ambrosini G, Nath AK, Sierra-Honigmann MR, et al. Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. J Biol Chem 2002;277: 34601-9.

[16] Grosfeld A, Zilberfarb V, Turban S, et al. Hypoxia increases leptin expression in human PAZ6 adipose cells. Diabetologia 2002;45: 527-30.

[17] Tschop M, Strasburger CJ, Hartmann G, et al. Raised leptin concentrations at high altitude associated with loss of appetite. Lancet 1998;352:1119-20.

[18] Tillmar L. Welsh N: Hypoxia may increase rat insulin mRNA levels by promoting binding of the polypyrimidine tract-binding protein (PTB) to the pyrimidine-rich insulin mRNA 3′-untranslated region. Mol Med 2002;8:263-72.

[19] Meissner U, Ostreicher I, Allabauer I, et al. Synergistic effects of hypoxia and insulin are regulated by different transcriptional elements of the human leptin promoter. Biochem Biophys Res Commun 2003; 303:707-12.

[20] Wolk R, Svatikova A, Nelson CA, et al. Plasma levels of adiponectin, a novel adipocyte-derived hormone, in sleep apnea. Obes Res 2005;13: 186-90.

[21] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548-56.

[22] Westerterp KR. Energy and water balance at high altitude. News Physiol Sci 2001;16:134-7.

[23] Bitar F, Feldbaum D, Kohman L, et al. Effect of early versus delayed hypoxic environment on neonatal rabbits. J Surg Res 1994;57:264-7.

[24] Bitar FF, Bitar H, ElSabban M, et al. Modulation of the ceramide content and lack of the apoptotic response in the chronically hypoxic rat bean. Pediatr Res 2002;51:144-9.

[25] Bennett BD, Solar GP, Yuan JQ, Mathias J, Thomas GR, Matthews W. A role for leptin and its cognate receptor in hematopoiesis. Curr Biol 1996;6:1170-80.

[26] Dagogo-Jack S. Uremic hyperleptinemia: adaptive or maladaptive? Kidney Int 1998;54:997-8.

[27] Bornstein SR, Licinio J, Tauchnitz R, Engelmann L, Negrao AB, Gold P, et al. Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal rhythm, in cortisol and leptin secretion. J Clin Endocrinol Metab 1998;83:280-3.

[28] Raff H, Bruder ED, Jankowski BM, et al. Effect of neonatal hypoxia on leptin, insulin, growth hormone and body composition in the rat. Horm Metab Res 2001;33:151-5.

[29] Baum D. Effect of acute hypoxia on circulating insulin levels. J Clin Endocrinol Metab 1969;29:991-4.

[30] Baum D, Porte Jr D. Beta adrenergic receptor dysfunction in hypoxic inhibition of insulin release. Endocrinology 1976;98:359-66.

[31] Mise H, Sagawa N, Matsumoto T, et al. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. J Clin Endocrinol Metab 1998;83:3225-9.

[32] Sagawa N, Yura S, Itoh H, et al. Role of leptin in pregnancy—a review. Placenta 2002;23 Suppl A:S80-6.

[33] Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292-5.

[34] Yasumasu T, Takahara A, Nakashima Y. Hypoxia inhibits leptin production by cultured rat adipocytes. Obes Res 2002;10:128.