Brain Regulation of Growth in Beef Calves

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Summary

Small peptide hormones produced in the lower part of the brain (hypothalamus) regulate episodic and basal secretion of hormones from the anterior pituitary gland that affect metabolism and growth in cattle. This study focused on long-term growth in young calves subjected to hypophysectomy (HYPOX), hypophyseal stalk transection (HST), and sham operation control (SOC). Crossbred (Hereford x Aberdeen Angus) and Hereford, and Aberdeen Angus calves were HYPOX (n = 5), HST (n = 5), or SOC (n = 8) at 146 days of age, whereas another group was HST (n = 5) or SOC (n = 7) at 273 days of age. Body weight was determined every 21 days from birth to 1008 days of age. From day 146-1008, growth was arrested ($P < 0.001$) in HYPOX (0.06 kg/day) compared with SOC (0.50 kg/day) calves. Growth continued but at a significantly lower rate ($P < 0.05$) in calves HST at 146 days (0.32 kg/day) and 273 days (0.32 kg/day) compared with SOC (0.50 kg/day). Although episodic growth hormone (GH) secretion was abolished and peripheral blood serum GH concentration remained consistently lower in HST calves (2.4 ng/ml) than in the SOC (5.5 ng/ml; $P < 0.01$), the calves continued to grow throughout 1008 days. Peripheral serum thyroid stimulating hormone (TSH) concentration was less ($P < 0.05$) in HST compared with SOC calves. There was an abrupt decrease ($P < 0.001$) in serum thyroxine (T_4) (4-fold) and triiodothyronine (T_3) (3-fold) concentration after surgery that remained to 360 days in HST compared with SOC calves. At sacrifice, pituitary gland weight was markedly reduced ($P < 0.001$) in HST (0.18 g/100 kg body weight) compared with SOC (0.55 g/100 kg body weight) calves. Histological examination of pituitary glands from HST calves indicated the persistence of secretory GH and TSH cells in the same areas of the anterior pituitary gland as SOC calves. Coronal sections of the gland revealed GH and TSH secreting cells in HST calves that were similar to the controls. These results indicate that long-term growth continues, but at a slower rate, after hypophyseal stalk transection of immature calves in spite of complete abolition of episodic GH secretion and consistently decreased basal secretion of GH, TSH, T_4, and T_3 compared with sham-operated animals. Growth was abolished after hypophysectomy of immature calves in which circulating GH and TSH was undetectable.

Introduction

Somatic growth in vertebrates is thought to be dependent on pituitary growth hormone (GH); without pituitary GH production or peripheral GH action, postnatal growth is severely stunted. For example, a deficiency in GH production or in the GH receptor (GHR) gene has been demonstrated to stunt growth. A notable exception is the guinea pig in which pituitary gland removal (hypophysectomy) does not alter the growth rate, and treatment with bovine GH (bGH) does not affect growth or increase insulin-like growth factor-I (IGF-I) levels. Hormones are generally released episodically, but evidence for the requirement of endogenous pulsatile GH secretion for growth in mammals is unknown. GH secretion in the guinea pig is pulsatile and controlled by endogenous GH-releasing hormone (GHRH), somatostatin (SRIH), and possibly a GH-releasing peptide receptor. GHR is expressed in various tissues and binds guinea pig GH; recent evidence focuses on an unusual binding specificity of guinea pig GH-binding protein (GHBP), being highly heterogeneous in molecular weight and binding affinities but more generalized in binding affinity for ovine- and bovine-GH, but not human GH. Although IGF-I and IGF-II are present in high concentration in guinea pig serum, hypophysectomy does not decrease nor does bGH or oGH treatment in such animals increase their production. The hypothalamus at the base of the brain regulates episodic GH secretion from the pituitary in part by its endogenous release of GHRH, SRIH, and possibly, a yet-unidentified GH secretagogue for which the receptor has been described. The neurophophyseal link between the hypothalamus and the pituitary is essential for transporting these releasing and inhibiting hormones. In the young animal, episodic GH secretion occurs during stages of rapid growth and wanes during maturity and senescence. Although aging animals and humans lack robust episodic GH secretion, the pituitary is fully capable of responding to GHRH or GH-secretagogue challenge with supra-physiological GH release.

Less clear is the role of episodic GH release in long-term growth. To our knowledge, no long-term studies have been carried out in the mouse, rat, hamster, rabbit or other species to determine the GH requirement for long-term growth after interruption of either episodic GH release or reduced basal GH secretion. This study focuses on long-term growth in calves subjected to either separation of the connecting link between the hypothalamus and pituitary (hypophyseal stalk transection) or hypophysectomy(1). The hypotheses to be tested were that cattle require endogenous GH secretion for growth but the episodic pattern for this hormone secretion is not essential for long-

term growth.

Materials and Methods

Animals

Fifteen male and 15 female crossbred (Hereford x Aberdeen Angus), and Hereford and Aberdeen Angus calves born at the Animal Reproduction Laboratory, Iowa State University, suckled their dam to 4 months of age. Calves were weighed at birth and 21-day intervals throughout the study.

Each animal was fitted with an indwelling catheter in a jugular vein before surgery, which was maintained for sequential bleeding. Blood (8 ml) was collected by venipuncture at 4-day intervals, centrifuged at 1500 x *g* and the serum stored at –20 °C until radioimmunoassay for GH, thyroid stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), and luteinizing hormone (LH).

Surgical procedures for hypophyseal stalk transection and hypophysectomy

Hypophyseal stalk transection (HST), hypophysectomy (HYPOX), and sham operation control (SOC) were performed as we previously described. For HST, the hypophyseal stalk was severed, and a nylon disc (8.0 mm diameter and 0.45 mm thickness) was inserted between the severed ends of the tubular stalk to prevent vascular regeneration. For HYPOX, the internal carotid and hypophyseal stalk were separated as described previously, the diaphragma sellae cut, and the pituitary gland removed with Hardy bayonet curettes and a Hardy pituitary spoon. The calves were monitored throughout the postoperative recovery period for changes in body temperature, respiration rate, and feed consumption.

Hormone radioimmunoassay

Serum GH was measured in 100 μl aliquots in duplicate by using highly purified bGH for labeling with ^{125}I by the chloramine T method, highly purified bGH for standards, and incubations at 4°C for 72 hours by procedures similar to those described previously. Assay sensitivity was 0.125 ng/tube. Intraassay and interassay coefficients of variance were 3.5% and 11.2%.

Serum TSH was measured in 200-μl aliquots in duplicate by using highly purified, bovine TSH for labeling with ^{125}I by the chloramine T method, purified bTSH for standards, and incubations at 4ºC by procedures similar to those described previously. Assay sensitivity ranged from 0.3-10 ng/tube. Intraassay and interassay coefficients of variance were 6.4% and 9.8%.

Thyroxine serum concentration was measured by using T_4-^{125}I immunoassay. Serum samples of 20 μl in duplicate were assayed with T_4 standards ranging from 2-32 μg/dl. Assay sensitivity was 2.5 ng/ml. Intraassay and interassay variances were 3.6% and 8.2%.

Triiodothyronine serum concentration was measured by using T_3-^{125}I immunoassay. Serum samples of 100 or 150 μl in duplicate were assayed with T_3 standards ranging from 25
to 800 ng/dl. Assay sensitivity was 4.5 ng/dl. Intraassay and interassay coefficients of variance were 2.5% and 6.0%.

Serum LH was measured in 200-μl aliquots in duplicate by using highly purified bovine LH (bLH, NIH) for labeling with ^{125}I by the chloramine T method and for standards (0.036-20 ng) similar to those procedures described previously. Assay sensitivity was 0.2 ng/ml. Intraassay and interassay coefficients of variance were 8.3% and 9.1%.

Histology

Postmortem examination of each animal confirmed the completeness of stalk transection. The nylon disc was in the proper location and had prevented vascular regeneration of the stalk in each calf. Furthermore, there was no development of a network of arterioles and venules for any revascularization between the hypothalamus and the pituitary gland in the HST calves. The pituitary gland from HST and SOC calves was cut transversely and fixed in Susa's solution for histological evaluation. Coronal sections of the glands were cut at 6 micrometers and stained with performic acid-Alcian blue-periodic acid-Schiff-orange G, whereas other sections were stained with hematoxylin and eosin. At sacrifice of HYPOX calves, the sella turcica was examined for remnants of pituitary tissue.

Statistical analyses

The experimental units in this study were the individual calves. Growth and hormone data were analyzed by a split-plot analysis using a one-way analysis of variance, and Student's *t* tests for continuous variables were used for comparisons between groups.

Results

Growth and food consumption

The weight of beef calves averaged 35 kg at birth and was 142 kg at 126 days and 208 kg at 252 days of age before surgery (Fig. 1 and 2). From days 146-1008, growth was arrested ($P < 0.001$) in HYPOX (0.06 kg/day) compared with SOC (0.50 kg/day) calves (Fig. 1). Growth continued but at a significantly lower rate ($P < 0.05$) in calves HST at 146 days (0.32 kg/day) and 273 days (0.32 kg/day) compared with the SOC (0.50 kg/day; Fig. 1 and 2). Long-term growth in HST calves to 1008 days of age was less in those HST at 146 days (432 kg body wt) than at 273 days (472 kg body wt) compared with the SOC (586 kg body wt; $P < 0.05$).

Figure 1. Growth in beef calves subjected to hypophysectomy (●), hypophyseal stalk transection (■) or sham operation (♦) at 146 days of age. Birth weights averaged 35 kg with subsequent body weights taken at 21-day intervals. Number of calves is indicated in parentheses. Values are mean ± SE.

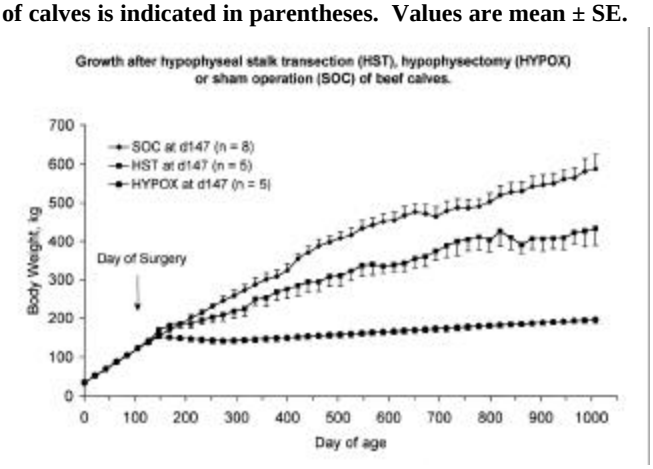
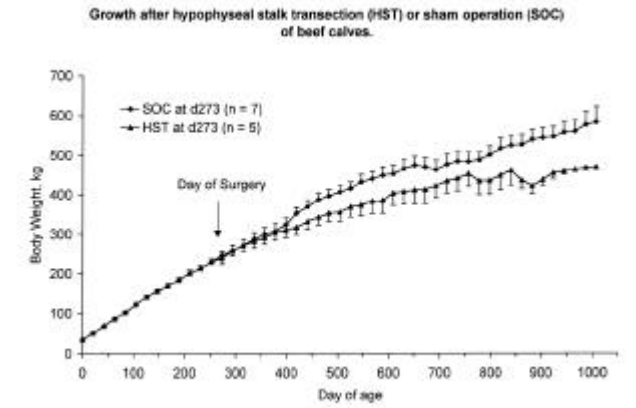


Figure 2. Growth in beef calves subjected to hypophyseal stalk transection (▲) or sham operation (●) at 273 days of age. Birth weights averaged 35 kg with subsequent body weights taken at 21-day intervals. Number of calves is indicated in parentheses. Values are mean ± SE.



GH peripheral serum concentration

From 72-146 days of age, GH serum concentration at 4-day intervals before surgery averaged 3-6 ng/ml (Fig. 3). After HST at day 146, GH decreased abruptly to 1.9 ng/ml and remained at this basal level to day 372. GH was consistently lower ($P < 0.01$) than in SOC calves, but within group there was no significant sex-related difference in serum GH concentration. Episodic GH secretion was abolished ($P < 0.001$) in HST compared with SOC calves as indicated by sequential blood sampling at 20-minute intervals throughout 8 hours (Fig. 4). Average GH serum concentrations remained consistently at the basal level of 2.4 ng/ml in HST compared with 5.5 ng/ml in SOC calves ($P < 0.01$).

Figure 3. GH concentration in peripheral serum at 4-day intervals before surgery ( ), and after HST (▲) and SOC (●) of beef calves to day 372 of age. Number of calves is indicated in parentheses. Values are mean ± SE.

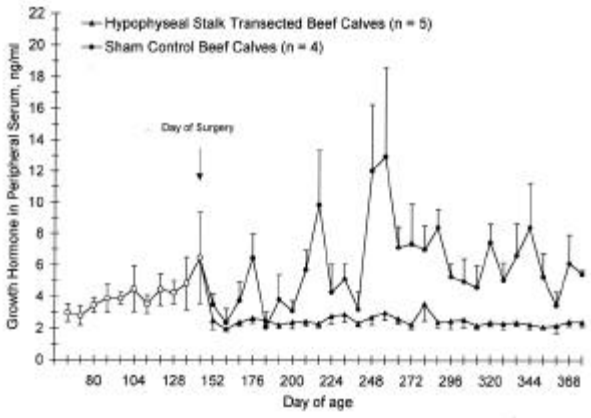
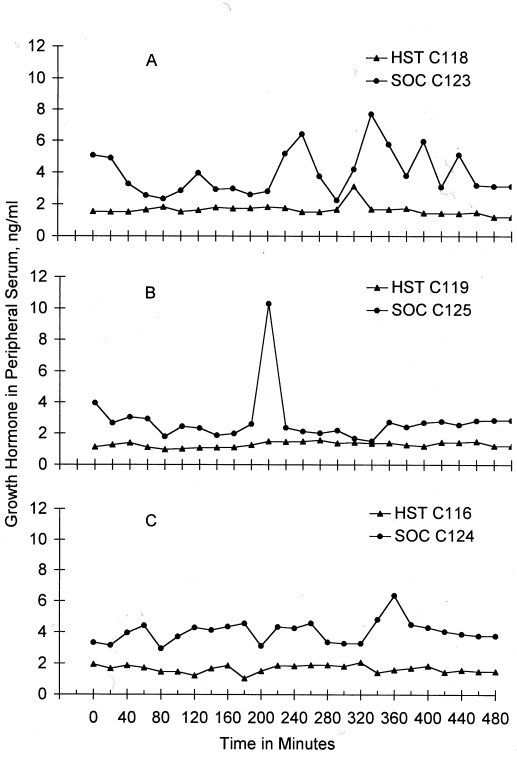


Figure 4. GH concentration in peripheral serum from sequential blood samples at 20-min intervals during 8 h in hypophyseal stalk transection (▲) and sham-operated control (●) beef calves. The number denotes the individual calf ear tag identification.



TSH, T_4, and T_3 peripheral serum concentration

From days 68-146 days of age before surgery, TSH peripheral serum concentration ranged from 5-7 ng/ml (Fig. 5). After HST, TSH concentration from days 146-360 was less ($P < 0.05$) than SOC calves. Thyroxine serum concentration ranged from 60-80 ng/ml before surgery in these young growing calves. Immediately following HST, T_4 decreased ($P < 0.001$) to less than 20 ng/ml and remained consistently at this basal level to day 360; T_4 ranged from 70-100 ng/ml throughout this period in the SOC calves. Triiodothyronine serum concentration ranged from 300-400 ng/dl before surgery. T_3 concentration abruptly decreased ($P < 0.001$) 3-fold to <100 ng/dl after HST and remained at this basal level to day 360; T_3 ranged from 250-300 ng/dl during this period in SOC calves (Fig. 5). There were no sex-dependent differences within groups of HST and SOC calves as related to TSH, T_4, and T_3 serum concentration.

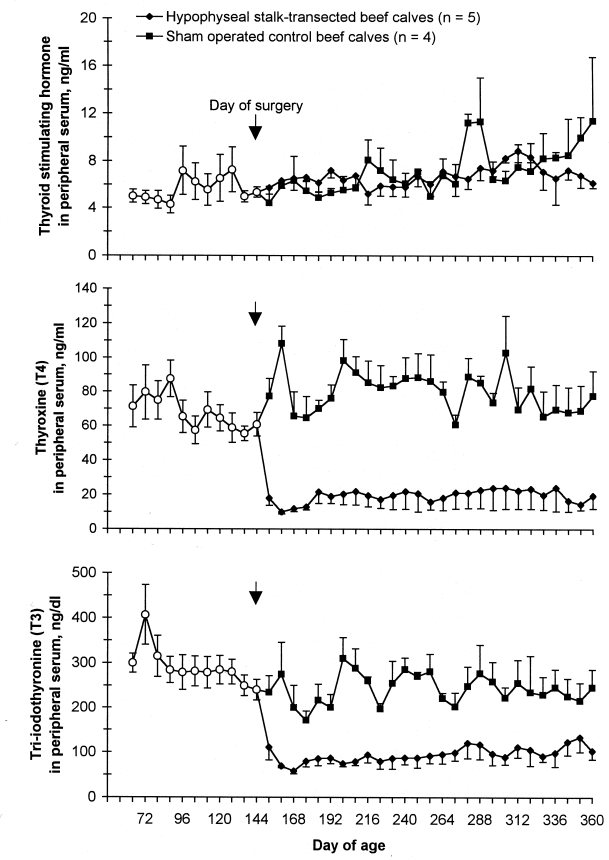


Figure 5. Thyroid stimulating hormone, thyroxine, and triodothyronine concentration in peripheral serum at 4-day intervals before surgery ( ), and after HST (♦) or SOC (■) of beef calves at 146 days to 360 days of age. Number of calves is indicated in parentheses. Values are mean ± SE.

LH peripheral serum concentration

Overall LH serum concentration as indicated by sequential blood sampling at 20-minute intervals throughout 8 hours was significantly decreased in HST compared with SOC calves (Table 1). Although baseline LH concentration remained similar in both groups, episodic LH secretion was abolished in HST compared with SOC calves, and was nondetectable in HYPOX calves.

Pituitary and thyroid histology

At sacrifice, pituitary gland weight of HST calves was 33% ($P < 0.01$) that of SOC animals (Table 2). In three HYPOX calves no pituitary remnants were found at sacrifice, whereas in the other two HYPOX animals the sella turcica contained approximately 10% scar tissue but no viable adenohypophyseal cells on histological examination.

Pituitary glands from HST calves indicated persistence of secretory cells in the same areas of the adenohypophysis as SOC. In coronal sections from HST and SOC calves stained with performic acid-Alcian blue-periodic acid (PAS)-Schiff-orange G, acidophils, basophils, and chromophobes were present. The severed ends of the hypophyseal stalk remained separated by the nylon disc in all HST animals.

Discussion

The main finding was that hypophyseal-stalk-transected calves continued growth but at a significantly lower rate than sham-operated controls even with the complete absence of episodic GH secretion, as well as a significantly lower basal blood concentration of GH and markedly decreased circulating thyroxine and triiodothyronine concentrations. In addition, the HST calves consumed significantly less daily feed than SOC that likely resulted from decreased appetite or feed to gain. At sacrifice, pituitary gland weight of HST calves was only 33% that of SOC. Pituitary histology indicated decreased cytoplasm of somatotrophs (GH cells) and thyrotrophs (TSH cells). Thyroid gland histology revealed greatly enlarged thyroid follicles bounded by cuboidal epithelium indicating a lack of thyroid hormone production and secretion in HST calves in contrast to vacuolated thyroid follicles bounded by columnar epithelium indicating normal thyroid hormone production and secretion in the SOC animals. Thus, in spite of compromised pituitary function in the HST calves, they continued to grow over a long period of time to a body weight at 1000 days that was 77% that of SOC animals.

The second finding was that cattle depend on pituitary GH secretion for somatic growth; hypophysectomy arrests long-term growth to 1000 days of age. GH concentration *in sera* from these hypophysectomized calves was below immunoassay sensitivity; long-bone growth, long winter and summer hair coat and diminished appetite reflected both GH deficiency and hypothyroidism. It is well known that GH in the pituitary, metabolic clearance rate (MCR), and
secretion all decrease significantly as cattle become larger. Release of GH from the bovine anterior pituitary gland is regulated by GHRH, SRIH, and likely by an as yet unidentified GH-secretagogue and other neuropeptides (i.e., galanin, neuropeptide Y) to modulate GH secretion.

Immediately following HST in calves, normal episodic secretion of GH and LH is abolished, whereas PRL secretion is consistently elevated over a period of 14 days. Thereafter, depressed growth rates, lack of onset of pubertal estrus, and decreased PRL blood concentration result in HST calves compared with SOC animals. Regardless, basal PRL serum concentration responds to seasonal changes, peaking in summer and exhibiting a nadir in winter, in both HST and SOC calves.

GH plays an important role in regulating metabolic changes necessary for growth and lactation in ruminants and in rats. Exogenous GH enhances retention of nitrogen, increases body weight gain in growing ruminants, and increases milk yield in dairy cows. A complex interaction between pituitary GH, the IGFs, their receptors, and their binding proteins in the regulation of statural growth applies in cattle and rats but remains unexplained in guinea pigs.

An age-dependent decrease occurs in overall and basal GH blood concentration and in GH amplitude and frequency of pituitary-intact crossbred beef calves from 5 to 15 months of age. In pituitary-intact castrate Holstein male cattle researchers compared the effect of administering GH intravenously for 10 days at a total dose of 48 μg/kg body weight daily either continuously, by 6 intravenous pulses daily, or a combination of continuous and pulse treatments, with vehicle controls on nitrogen retention. Both area-under-the-curve GH and nitrogen retention were increased by GH administration during the 10-day period compared with vehicle treated controls. There were no significant differences in nitrogen retention, however, as related to either continuous or pulse intravenous administration of GH in these cattle. A longer term study with crossbred beef steers intramuscularly injected with GH at 22.8 mg/day for 56 days resulted in a significant increase in protein gain in

muscles of the carcass as well as non-carcass components of the empty body compared with vehicle injected controls. GHRH intravenously injected every 4 hours for 10 days significantly increased GH pulse amplitude, and a trend toward greater nitrogen retention, but this was not statistically significant compared with vehicle treated control beef calves. Thus, it seems that raising the overall circulating basal GH concentration rather than GH pulse amplitude is required for positive nitrogen retention and growth in cattle. Other research findings in intact cattle support our hypothesis in that growth (positive nitrogen retention) was related to raising the overall basal GH concentration rather than an enhanced episodic pattern of hormone secretion.

Implications

The results from the present study in cattle show that long-term growth is sustained by non-episodic basal secretion of GH after hypophyseal stalk transection, but absence of GH after hypophysectomy completely arrests growth. Thus, this ruminant species requires at least a modest basal level of GH secretion for statural growth unlike the guinea pig, a herbivore that is developmentally mature at birth, in which growth for a prolonged period continues unabated after hypophysectomy. In contrast, the rat, developmentally immature at birth and omnivorous after weaning, requires GH for growth; hypophysectomy arrests growth, but it is unknown whether non-episodic endogenous basal GH secretion sustains long-term growth after HST in that species. From these results it seems that increasing circulating GH blood concentrations or augmenting endogenous basal secretion of the hormone could alter metabolism and nitrogen deposition for increased growth in cattle.

Table 1. Serum LH concentration in SOC, HST, and HYPOX beef calves.

	SOC	HST	HYPOX
No. of calves	5	5	5
Overall concentration, ng/ml	0.90 ± 0.18	0.28 ± 0.02[b]	ND [a,c]
Baseline concentraiton, ng/ml	0.25 ± 0.01	0.24 ± 0.02	ND [a,c]
Pulse amplitude, ng/ml	4.4 ± 0.6	ND [a,c]	ND [a,c]
Pulse frequency/8 hours	3.4 ± 0.5	ND [a,c]	ND [a,c]

Values are mean ± SE.
[a]Not detectable (<0.2 ng/ml).
[b]P < 0.05 vs SOC.
[c]P < 0.01 vs. SOC.

Table 2. Bovine pituitary, adrenal, and thyroid gland development after hypophyseal stalk transection, hypophysectomy, or sham operation.

					Thyroid glands				
	No. of calves	Pituitary gland wt (g)	Pituitary gland g/100 kg body wt	Adrenal glands g/100 kg body wt	wt (g)	g/100kg body wt	Epithelial cell diameter μm	Vacuolization of follicles %	Vacuolization of follicles Rank[a]
SOC	10	2.65 ± 0.189	0.54 ± 0.025	4.70 ± 0.170	25.2 ± 3.39	4.3 ± 0.58	9.4 ± 0.8	93 ± 4	4.5 ± 0.2
HST	10	1.05 ± 0.072[c]	0.18 ± 0.010[c]	5.10 ± 0.290	26.9 ± 3.03	4.8 ± 0.54	5.9 ± 0.4[b]	56 ± 8[c]	2.0 ± 0.4[c]
HYPOX	5			2.66 ± 0.215[b]	3.9 ± 0.35[c]	2.5 ± 0.22[b]	5.9 ± 0.3[b]	73 ± 7[b]	2.0 ± 0.4[c]

Values are mean ± SE.
[a]Scale from 1 to 5 for slight to extensive vacuolization.
[b]P < 0.05 vs SOC.
[c]P < 0.01 vs. SOC.