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INFLUENCE OF DIETARY ENERGY INTAKE ON THE HYPOTHALAMIC-PITUITARY TESTICULAR AXIS OF PREPUBERTAL BRAHMAN BULLS

C. J. Nolan, D. A. Neuendorff, R. W. Godfrey, P. G. Harms, N. H. McArthur, T. H. Welsh, Jr. and R. D. Randel

SUMMARY

Limiting dietary energy intake will increase the age at which a bull reaches puberty. Twelve prepubertal half-sib Brahman bulls were fed individually to gain either .2-.6 lb/hd/d (moderate gain; MG) or 1.6-2.2 lb/hd/d (high gain; HG). Serum samples were obtained at four ages for analysis of luteinizing hormone (LH), growth hormone (GH) and testosterone concentrations. Additionally, following slaughter gonadotropin releasing hormone (GnRH) secretion by the median eminence was examined *in vitro*. Serum LH increased as the bulls aged, but was not different between MG and HG bulls. Serum GH was influenced with both age and energy intake. Serum testosterone was increased by increased dietary energy intake and was also increased with increased age. There was also an increase in *in vitro* GnRH released by the meidan eminence due to increased dietary energy intake. These data suggest that the differences in physiological age due to dietary energy intake also alter the endocrine function of the hypothalamic-pituitary-testicular axis.

INTRODUCTION

The objectives of this study were 1) to examine the influence of dietary energy intake on serum luteinizing hormone, growth hormone and testosterone and 2) to examine the effect of energy intake on the *in vitro* release of gonadotropin releasing hormone from the median eminence.

PROCEDURES

Twelve half-sib prepubertal Brahman bulls (373 to 433 lb; 253 to 285 d of age) were randomly assigned, within age and weight pairs, to dietary treatment groups. Bulls were individually fed each morning to gain either .2 to .6 (moderate gain; MG) or 1.6 to 2.2 (high gain; HG) lb/hd/d. Blood samples were obtained every 20 min for 6 h at 4 ages (days 0, 56 and 112 on feed and following appearance of first motile spermatozoa [FS], in the ejaculate of the HG bull in an age x sire group). Serum was harvested and stored until analysis for luteinizing hormone (LH), growth hormone (GH) and testosterone (T). Following FS, bulls were slaughtered and the median eminence placed in an *in vitro* perifusion system and the release of gonadotropin releasing hormone (GnRH) quantitated.

RESULTS

The secretion of LH was increased (P<.001) as the bulls increased in age (Table 1). All characteristics of LH secretion evaluation (mean, basal, pulse height and amplitude and total LH secreted/6h) except pulse frequency increased as the bulls increased in age. There were, however, no effects of dietary energy intake on serum LH secretion (P>.10), nor were there any interactions between age and energy intake (P>.10).

Serum GH is known to respond to whole meal feeding, therefore the 6 h blood sampling period was divided into the 3 h period immediately following feeding and a remaining 3 h period to examine "normal" GH secretion. The height (P<.02) and amplitude (P<.06) of the GH peak induced by feed intake (Table 2) was increased by increased age. The greatest increases were observed after the bulls had been on feed 112 d (approximately 12 months of age) and were greater than twice that observed at the onset of the study (approximately 8 months of age). There was no effect of dietary energy intake on feeding-induced GH secretion (P>.10), nor were there any age x energy interactions (P>.10). The "normal" secretion characteristics were influenced by both age and dietary energy intake (Table 3). Increased age resulted in increases in both basal (P<.02) and the total amount of GH secretion (P<.001), but both were not affected by dietary energy intake (P>.10). Dietary energy intake did however, influence mean GH (P<.03) and both GH pulse height and amplitude (P<.06). For all three of these characteristics enhanced dietary energy resulted in a lower amount of GH secretion, while there was no effect of age on these characteristics (P>.10).

Taken together with the data for LH secretion these results indicate direct effects of both age and dietary energy intake on pituitary hormone secretion. These influences on pituitary function may be one of the potential factors regulating the onset of puberty in Brahman bulls.

All characteristics of serum testosterone secretion, except pulse frequency, were increased by both increased age and dietary energy intake (Table 4). Increased age increased ($P<.001$) mean, basal, pulse height and amplitude and total testosterone secreted between 4 and 5-fold from the onset of the study (approximately 8 months of age) to the end of the study (approximately 13 months of age). Providing increased dietary energy resulted in enhanced testosterone secretion ($P<.01$). Testosterone secretion characteristics in HG bulls were approximately twice that of MG bulls. Interactions between age and dietary energy intake were found to influence mean ($P<.002$), basal ($P<.02$) and total testosterone secreted ($P<.02$). For all three characteristics it was observed that providing dietary energy to enhance growth increased testosterone secretion more rapidly compared to slower gaining bulls. These data for testosterone secretion indicate that the testis may be dramatically influenced by the level of dietary energy intake and therefore impact upon the age at puberty.

To examine hypothalamic function the median eminence (ME) was perifused and at three times challenged with a high concentration of potassium (K^+) to induce GnRH release (Table 5). Basal GnRH release was enhanced in HG compared to MG bulls ($P<.05$; Table 4). There were no differences, however, in responsiveness to K^+ due to dietary energy intake ($P>.10$). There were no differences in GnRH concentration within the ME due to dietary energy ($P>.10$).

In conclusion, based on the results from this study and the companion study, it appears that dietary energy intake influences all portions of the hypothalamic-pituitary-testicular axis. Increasing dietary energy intake to realize weight gain at optimal (1.6 to 2.2 lb/d) compared to suboptimal (.2 to .6 lb/d) rate enhanced in vitro GnRH secretion from the ME and testosterone secretion by the testes. Pituitary LH secretion was not influenced by dietary energy, while GH secretion was elevated by limiting energy intake. These data indicate that additional studies are needed to identify the relationship(s) between growth, nutrient intake and the onset of puberty in Brahman bulls.

TABLE 1. INFLUENCE OF AGE AND DIETARY ENERGY INTAKE ON SERUM LUTEINIZING HORMONE (LH) CONCENTRATIONS[a]

	Days on Feed				
	0	56	112	FS	Mean
Mean LH (ng/ml)					
Moderate gain	.72±.58	1.64±.45	2.96±.50	3.67±.61	2.25±.25
High gain	.50±.70	1.83±.45	3.22±.42	3.78±.52	2.33±.24
Mean	.61±.45[b]	1.70±.30[c]	3.09±.33[d]	3.73±.30[d]	
Basal LH (ng/ml)					
Moderate gain	.20±.25	.48±.19	.79±.21	1.05±.19	.64±.10
High gain	.14±.29	.53±.16	1.02±.18	1.26±.16	.72±.10
Mean	.16±.19[b]	.50±.13[b]	.90±.14[c]	1.16±.13[c]	
LH pulse height (ng/ml)					
Moderate gain	2.01±2.62	4.69±1.84	7.94±2.40	11.52±2.12	6.54±1.17
High gain	2.25±2.12	4.69±2.12	8.37±2.00	12.29±1.84	6.12±1.15
Mean	2.17±2.16[b]	4.69±1.40[b,c]	8.15±1.56[c]	11.91±1.40[d]	
LH pulse amplitude (ng/ml)					
Moderate gain	1.71±2.62	4.12±2.03	7.15±2.28	10.47±1.34	5.86±1.12
High gain	2.15±3.18	4.20±1.75	7.35±1.92	11.04±1.75	5.46±1.10
Mean	2.04±2.06	4.16±1.34[b,c]	7.25±1.49[c]	10.75±1.34[d]	
LH pulse number/6 h					
Moderate gain	2.0 ± .8	2.2 ± .4	2.5 ± .5	2.7 ± .4	2.3 ± .3
High gain	2.8 ± .7	2.3 ± .5	2.2 ± .5	2.0 ± .5	2.3 ± .3
Mean	2. 4± .5	2.2 ± .3	2.3 ± .4	2.3 ± .3	
Total LH released/6 h ($ng \cdot min^{-1} \cdot ml^{-1}$)					
Moderate gain	351.5±397.5	580.7±38.9	2234.9±439.0	2309.9±389.6	1254.5±214.6
High gain	375.4±398.1	652.2±337.4	1157.2±368.9	1359.5±357.4	834.9±211.1
Mean	366.1±396.2	616.5±257.7[b]	1696.1±286.8[c]	1834.7±257.7[c]	

[a]Values are LS means ± SEM.
[b,c,d]Different superscripts within rows indicate differences due to age (P<.001).

TABLE 2. INFLUENCE OF AGE AND DIETARY ENERGY INTAKE ON FEEDING-INDUCED GROWTH HORMONE (GH) RELEASE[a]

	Days on feed				
	0	56	112	FS	Mean
Peak height (ng/ml)					
Moderate gain	5.10 ± 2.21	8.08 ± 2.21	16.14 ± 2.50	11.74 ± 1.87	10.30 ± 1.20
High gain	7.52 ± 1.96	9.66 ± 1.96	11.44 ± 1.76	7.72 ± 1.95	9.10 ± 0.90
Mean	6.31 ± 1.48[b]	8.87 ± 1.48[b]	13.79 ± 1.53[c,d]	9.72 ± 1.35[d]	
Peak amplitude (ng/ml)					
Moderate gain	4.72 ± 2.21	7.39 ± 2.21	14.57 ± 2.51	8.55 ± 1.86	8.80 ± 1.22
High gain	6.86 ± 1.97	8.21 ± 1.96	9.13 ± 1.76	6.21 ± 1.95	7.60 ± 0.94
Mean	5.79 ± 1.48[e]	7.80 ± 1.48[e]	11.85 ± 1.53[f]	7.38 ± 1.35[e]	
Time to peak (min)					
Moderate gain	78.7 ± 17.7	86.7 ± 17.7	100.7 ± 20.1	86.7 ± 15.0	88.2 ± 9.7
High gain	102.7 ± 15.8	66.2 ± 15.8	59.8 ± 14.1	54.6 ± 15.6	70.8 ± 7.5
Mean	90.7 ± 11.8	76.4 ± 11.8	80.3 ± 12.3	70.6 ± 10.8	

[a]Values are LS Means ± SEM.
[b,c,d]Different superscripts within rows indicate differences due AGE (P<.02).
[e,f]Different superscripts within rows indicate differences due to AGE (P<.06).

TABLE 3. INFLUENCE OF AGE AND DIETARY ENERGY INTAKE ON GROWTH HORMONE (GH) SECRETION[a]

	Days on feed				
	0	56	112	FS	Mean
Basal GH (ng/ml)					
Moderate gain	.71 ± .55	.99 ± .55	1.74 ± .62	1.66 ± .28	1.66 ± .28
High gain	.61 ± .47	1.96 ± .47	2.24 ± .53	1.59 ± .47	1.59 ± .24
Mean	.66 ± .36[b]	1.47 ± .36[b,c]	1.99 ± .40[c,d]	2.36 ± .36[d]	
Mean GH (ng/ml)					
Moderate gain	4.58 ± .70	4.16 ± .70	4.58 ± .70	5.86 ± .79	4.80 ± .36[e]
High gain	2.51 ± .61	2.48 ± .61	3.79 ± .66	3.54 ± .61	3.08 ± .31[f]
Mean	3.55 ± .46	3.31 ± .46	4.19 ± .48	4.70 ± .50	
GH pulse number/3h					
Moderate gain	1.2 ± .3	1.0 ± .3	0.8 ± .3	0.8 ± .3	1.0 ± .1
High gain	1.1 ± .2	1.0 ± .2	1.1 ± .3	1.4 ± .2	1.1 ± .1
Mean	1.1 ± .2	1.0 ± .2	0.9 ± .2	1.1 ± .2	
GH pulse height (ng/ml)					
Moderate gain	11.71 ± 1.71	12.02 ± 11.93	10.21 ± 2.24	10.03 ± 1.96	10.99 ± .99[g]
High gain	7.10 ± 1.62	7.04 ± 1.82	7.87 ± 2.03	9.98 ± 1.62	8.00 ± .89[h]
Mean	9.40 ± 1.18	9.53 ± 1.33	9.04 ± 1.51	10.01 ± 1.27	
GH pulse amplitude (ng/ml)					
Moderate gain	11.00 ± 1.49	11.07 ± 1.67	8.04 ± 1.94	6.96 ± 1.70	9.27 ± .86[g]
High gain	6.24 ± 1.41	5.04 ± 1.58	6.42 ± 1.76	7.11 ± 1.41	6.20 ± .78[h]
Mean	8.62 ± 1.02	8.06 ± 1.15	7.23 ± 1.31	7.04 ± 1.10	
Total GH secreted/6h ($ng \cdot min^{-1} \cdot ml^{-1}$)					
Moderate gain	1234.6 ± 257.4	1076.3 ± 257.4	1949.2 ± 289.7	2364.2 ± 257.4	1656.1 ± 132.9
High gain	802.8 ± 222.9	1102.4 ± 222.9	1804.8 ± 243.2	1316.2 ± 222.9	1255.5 ± 114.1
Mean	1018.7 ± 170.3[i]	1089.3 ± 170.3[i]	1876.9 ± 189.1[j]	1840.2 ± 170.3[j]	

[a]Values are LS Means ± SEM.
[b,c,d]Different superscripts within rows indicate differences due to age (P<.02).
[e,f]Different superscripts within columns indicate differences due to dietary energy intake (P<.03).
[g,h]Different superscripts within rows indicate differences due to dietary energy intake (P<.06).
[i,j]Different superscripts within rows indicate differences due to age (P<.001).

TABLE 4. INFLUENCE OF AGE AND DIETARY ENERGY INTAKE ON SERUM TESTOSTERONE CONCENTRATIONS[a]

	Days on feed				
	0	56	112	FS	Mean
Mean testosterone (ng/ml)					
Moderate gain	.25 ± .5	.39 ± .15	.44 ± .16	.71 ± .15	.45 ± .08[b]
High gain	.24 ± .15	.50 ± .15	1.15 ± .16	1.55 ± .5[e]	.86 ± .08[c]
Mean	.24 ± .10[h]	.45 ± .10[h]	.80 ± .11[i]	1.13 ± .10[i]	
Basal testosterone (ng/ml)					
Moderate gain	.18 ± .15	.39 ± .15	.44 ± .16	.71 ± .15±	.27 ± .04[d]
High gain	.17 ± .08	.28 ± .08	.62 ± .09	.93 ± .08	.50 ± .04[e]
Mean	.18 ± .06[h]	.28 ± .06[h]	.44 ± .06[i]	.65 ± .06[i]	
Testosterone pulse height (ng/ml)					
Moderate gain	.50 ± .51	.99 ± .51	1.47 ± .63	2.17 ± .46	1.28 ± .27[b]
High gain	.85 ± .51	1.98 ± .51	4.22 ± .63	3.98 ± .46	2.76 ± .27[c]
Mean	.67 ± .36[h]	1.48 ± .36[h]	2.85 ± .45[i]	3.07 ± .33[i]	
Testosterone pulse amplitude (ng/ml)					
Moderate gain	.31 ± .44	.72 ± .45	1.21 ± .55	1.79 ± .41	1.01 ± .23[f]
High gain	.70 ± .45	1.68 ± .45	3.49 ± .55	3.05 ± .41	2.23 ± .23[g]
Mean	.50 ± .31[h]	1.20 ± .32[h]	2.35 ± .39[i]	2.42 ± .29[i]	
Testosterone pulse number/6h					
Moderate gain	1.0 ± .2	1.1 ± .2	.71 ± .2	1.0 ± .2	1.0 ± .1
High gain	.9 ± .2	1.1 ± .2	.71 ± .2	1.0 ± .2	.9 ± .1
Mean	.9 ± .1[h]	1.1 ± .1[h]	.7 ± .1[i]	1.0 ± .1[h]	
Total testosterone release/6h ($ng \cdot min^{-1} \cdot ml^{-1}$)					
Moderate gain	82.5 ± 51.6	136.1 ± 51.6	147.7 ± 56.3	244.3 ± 51.6	152.6 ± 26.4[b]
High gain	85.8 ± 51.6	168.4 ± 51.6	404.0 ± 56.3	540.1 ± 51.6[k]	299.6 ± 26.4[c]
Mean	84.1 ± 36.5[h]	152.3 ± 36.5[h]	275.8 ± 39.8[i]	392.2 ± 36.5[i]	

[a]Values are LS Means ± SEM.
[b,c]Different superscripts within columns indicate differences due to dietary energy (P<.005).
[d,e]Different superscripts within columns indicate differences due to dietary energy (P<.002).
[f,g]Different superscripts within columns indicate differences due to dietary energy (P<.008).
[h,i]Different superscripts within rows indicate differences due to age (P<.0001).

TABLE 3. INFLUENCE OF DIETARY ENERGY INTAKE ON MEDIAN EMINENCE GnRH RELEASE IN VITRO AND GONADOTROPIN RELEASING HORMONE (GnRH) CONCENTRATION[a]

	Dietary energy	
	Moderate gain	High gain
Basal GnRH ($pg \cdot mg^{-1} \cdot ml^{-1}$)	1.10±.11	1.14±.10
K+-induced GnRH peak height ($pg \cdot mg^{-1} \cdot ml^{-1}$)	3.31±.40	3.40±.35
K+-induced GnRH peak amplitude ($pg \cdot mg^{-1} \cdot ml^{-1}$)	2.15±.35	2.00±.32
Area under the K-induced GnRH peak (pg·min/mg·ml)	122.2±13.8	138.8±12.3
GnRH concentration (ng/mg)	.42±.10	.45±.10

[a]Values are LS means ± SEM.