Nutrition 30 (2014) 890–896

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Feeding prepubescent gilts a high-fat diet induces molecular changes in the hypothalamus-pituitary-gonadal axis and predicts early timing of puberty

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ARTICLE INFO

Article history:
Received 29 July 2013
Accepted 25 December 2013

Keywords:
High-fat diet
Hormone
Gene expressions
Puberty
Gilts

ABSTRACT

Objective: The onset of puberty in females has been occurring earlier over the past decades, presumably as a result of improved nutrition in developed countries. However, the underlying molecular mechanisms responsible for the early attainment of puberty as a result of nutrition fortification remain largely unknown. The aim of this study was to evaluate the hormone and gene expression changes in prepubescent gilts fed a high-fat diet to investigate whether these changes could predict the early timing of puberty.

Methods: Forty gilts were fed a daily basal diet (LE) or a basal diet with an additional 270 g/d or 340 g/d of fat (HE) during the prepubescent phase. Blood samples were collected during the prepubescent phase to detect hormone secretion changes in insulin-like growth factor-1, kisspeptin, estradiol, progesterone, and leptin. The gene expressions at the hypothalamus–pituitary–gonadal axis were examined on day 73 of the experiment (average age on day 177) during the prepubescent phase.

Results: An HE diet resulted in accelerated body weight gain and back-fat thickness at the P_2 point compared with LE gilts during the prepubescent phase. Gilts that were fed HE diets attained puberty 12 d earlier than LE gilts, and a larger proportion of HE gilts reached puberty at day 180 or 190 of age. A postmortem analysis revealed a promoted development of the uterus and ovary tissue that was characterized by a 53.7% and 29.5% increase in the uterine and ovary weight, respectively, and an increased length of the uterine horn and oviduct tissue in HE gilts. Real-time quantitative polymerase chain reaction revealed that HE gilts had higher *Kiss-1*, G protein-coupled receptor 54, gonadotropin-releasing hormone and estrogen receptor α mRNA expression levels in the hypothalamic anteroventral periventricular nucleus; the leptin receptor mRNA expression level was higher in the hypothalamic arcuate nucleus and ovary tissue; the insulin-like growth factor-1 receptor expression was higher in the pituitary and ovary tissues, and the follicle-stimulating hormone and luteinizing hormone mRNA expression levels were higher in the pituitary gland.

Conclusion: These data showed that the consumption of additional fat can facilitate early attainment of puberty, which can be predicted by the changes in secreted hormones and gene expression in the hypothalamus-pituitary-gonadal axis.

Introduction

The onset of puberty in females, as measured by the age at menarche, is estimated to have advanced by 6 to 12 mo per 100 y between the 18th and 21st centuries in several northern European countries [1]. This declining age of puberty has been

This work was supported by the National Natural Science Foundation (30871804) of PR China and Program for Changjiang Scholars and Innovative Research Team in University (IRT13083).

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0899-9007/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2013.12.019

attributed to accelerated growth resulting from improved nutrition, e.g., obese girls tend to mature earlier than normal or thin girls [2–4]. Thus, the accelerated growth rate due to nutrition fortification seems to be a more important index in predicting the early onset of puberty [5]. Although body condition and the timing of puberty show a strong link in developing girls, the underlying mechanism remains largely unknown.

Puberty is a complex biological process that involves the secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus, sexual development, adrenal maturation, pubertal development, and gametogenesis. Notably, the secretion of GnRH by the hypothalamus represents the first known step in the reproductive cascade to initiate the activation of pituitary and gonadal function. Therefore, understanding the neuroendocrine control of GnRH secretion may provide insight into the normal reproduction or disorder of the pubertal process. Recently, pharmacologic and genetic studies revealed that *GPR54*, a G protein-coupled receptor gene, may act as a gatekeeper for normal GnRH physiology and puberty [6]. Kisspeptins, which are encoded by the metastasis suppressor gene *Kiss-1*, are a natural ligand for *GPR54* to elicit a GnRH surge and puberty onset [7,8]. *Kiss-1* and *GPR54* may be involved in the early timing of puberty due to accelerated growth. Thus, their gene expression levels represent an interesting research topic.

In the present study, developing gilts were fed an oil-rich diet to induce hormone and molecular changes in the hypothalamus, pituitary, and gonadal tissues. These changes were used to assess whether hormone and molecular changes could predict the early onset of puberty.

Materials and methods

All experimental procedures were approved by the Animal Care and Use Committee of Sichuan Agricultural University.

Animals and diets

Forty Landrace × Yorkshire crossbred gilts of initially similar body weight (55 ± 1.5 kg) and age (104 ± 2 d) were paired and fed one of two nutritional regimens. Twenty gilts were fed basal diets (LE) formulated to provide 3.22 Mcal/kg digestible energy, 19.1% crude protein and 3.25% ether extract, as recommended by the National Research Council [9]. LE gilts in the 55- to 100-kg phase and 100-kg to puberty phase were fed a 1.8- and 2.1-kg basal diet, respectively. The other 20 gilts (HE) were fed basal diets containing an additional 270 or 340 g/d fat during these two phases, respectively. Finally, due to additional fat intake, HE gilts consumed 40.7% and 44.1% more digestible energy than LE gilts during the 55- to 100-kg phase and 100-kg to puberty phase, respectively. The intakes of protein, minerals, and vitamins were similar for both gilts (Table 1). Gilts were housed individually (2 m × 0.8 m) in a breeding facility and fed twice daily at 0830 and 1430 h, and water was provided ad libitum. The environment temperature was controlled at 20°C to 24°C. Another subgroup of gilts was simultaneously reared and fed LE or HE diets and used to substitute gilts that were culled because of lameness or illness. If gilts in the LE or HE were culled, the corresponding paired gilts were discarded as well.

Table 1
Daily nutrient intake in gilts fed LE or HE diets

	55–100 kg		100 kg to puberty	
	LE	HE	LE	HE
Intake of basal diet (g/d)	1800	1800	2100	2100
Oil intake (g/d)	0	270	0	340
Total feed intake (g/d)	1800	2070	2100	2440
Calculated daily nutrient intake (g/d)				
Digestible energy (Mcal/d)	5.80	8.16	6.76	9.74
Crude protein	344	344	401	401
Starch	842	842	983	983
Lipid	59	329	68	408
Crude fiber	51	51	59	59
Lysine	16	16	19	19
Calcium	17	17	20	20
Phosphorus	13	13	15	15
Available phosphorus	9	9	10	10

HE, gilts fed basal diets with additional oil supplements; LE, gilts fed basal diets

Determination of body weight and back-fat thickness

The fasted body weights were determined in the morning before feeding at the beginning of the experiment and at days 52, 59, 66, and 73 of the experiment. Back-fat thickness was measured 6 cm from the midline of the last rib (P_2 thickness) using a Lean Meater (Renco-Lean Meater, Minneapolis, MN, USA) at beginning of experiment and at days 52, 59, 66, and 73 of the experiment.

Estrous detection

Estrous was not induced by exogenous hormone administration for these gilts. Estrous was carefully checked to ensure that puberty was detected. All gilts were exposed (with fence) to mature boars to encourage pubertal estrous. Estrous was detected by only one experienced stockperson based on behavioral and vulvar characteristics. The appearance of a pink vulva and vaginal orifice mucous were important signs of estrous initiation, while standing still under applied back pressure (standing reflection) was used as an important behavioral criterion to establish onset of estrous. Age at puberty was recorded on the day of estrous.

Tissue sample collection

In the present study, gilts began to express estrous at day 72 of the experiment. To examine nutrition-induced molecular changes in the hypothalamus–pituitary–gonadal axis, 5 pair-fed prepubescent gilts from corresponding LE and HE groups were sacrificed to collect tissue samples from the hypothalamus, pituitary, and gonadal tissues.

The brains were removed from the skull immediately after sacrifice, and excess tissues were removed. The hypothalamus samples were collected as previously described [10]. Briefly, the hypothalamus was dissected along the following boundaries: Laterally 8 mm from either side of the third ventricle, longitudinally 8 mm from the optic chiasm to the posterior border of the mammillary bodies, and 20 mm above the top surface of the thalamus. The anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) of the hypothalamus were dissected as previously described [10].

The uterus, oviduct, and ovary tissues were removed from the abdominal cavity immediately after sacrifice and pruned for excess intestinal and other viscera tissues. The uterus was weighed, and the length of the uterus and oviduct were measured. These tissues were washed three times with phosphate-buffered solution followed by drying with paper towels before weighing.

All hypothalamic, pituitary, and ovary samples were washed with phosphate-buffered solution, dried with paper, frozen in liquid nitrogen, and stored at −70°C for the future analysis of gene expressions.

Determination of hormone concentrations

To explore the hormone secretion changes of gilts fed an oil-added diet, 10 mL blood samples were collected from both LE and HE gilts by jugular puncture at the beginning of the experiment and at days 52, 59, 66, and 73 of the experiment during the prepubescent phase. The blood samples were centrifuged at 2400 g for 15 min at 4°C to collect serum and stored at −20°C for the future analysis of hormone concentrations.

An enzyme-linked immunosorbent assay was used to determine the concentrations of insulin-like growth factor (IGF)-1 (commercial kit from Alpco Diagnostics, Windham, NH, USA), kisspeptin (commercial kit from Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA), leptin (commercial kit from R&D, Minneapolis, MN, USA), estradiol (commercial kit from R&D), and progesterone (P4; commercial kit from R&D). The detection limits of IGF-1, kisspeptin, estradiol, P4, and leptin were 0.09 ng/mL, 0.01 ng/mL, 1.1 pg/mL, 0.01 ng/mL, and 0.01 ng/mL, respectively. The intra- and interassay coefficients of variation were 5.3% and 5.9%, 5.0%, and 4.7%, 5.6% and 6.9%, 5.7% and 6.8%, 5.4% and 6.1%, for IGF-1, kisspeptin, estradiol, P4, and leptin, respectively.

Gene expressions

Gene expression levels were detected using a real-time quantitative polymerase chain reaction (PCR). The primers for the target genes are presented in Table 2. The mRNA expression levels of *Kiss-1*, *GPR54*, *GnRH*, follicle-stimulating hormone (*FSH*), luteinizing hormone (*LH*), IGF-1 receptor (*IGF-1 R*), insulin receptor (*Ins-R*), leptin receptor (*Ob-R*), estrogen receptor α (*ERα*) and progesterone (*P4 R*) were determined with real-time quantitative (q)PCR. Total RNA was isolated from tissue samples using Trizol Reagent (Invitrogen, Carlsbad, CA, USA)