Mechanism of Programmed Obesity in Intrauterine Fetal Growth Restricted Offspring: Paradoxically Enhanced Appetite Stimulation in Fed and Fasting States

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Abstract

We have shown that intrauterine fetal growth restriction (IUGR) newborn rats exhibit hyperphagia, reduced satiety, and adult obesity. Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a principal metabolic regulator that specifically regulates appetite in the hypothalamic arcuate nucleus (ARC). In response to fasting, upregulated AMPK activity increases the expression of orexigenic (neuropeptide Y [NPY] and agouti-related protein [AgRP]) and decreases anorexigenic (proopiomelanocortin [POMC]) peptides. We hypothesized that IUGR offspring would exhibit upregulated hypothalamic AMPK, contributing to hyperphagia and obesity. We determined AMPK activity and appetite-modulating peptides (NPY and POMC) during fasting and fed conditions in the ARC of adult IUGR and control females. Pregnant rats were fed ad libitum diet (control) or were 50% food restricted from gestation day 10 to 21 to produce IUGR newborns. At 10 months of age, hypothalamic ARC was dissected from fasted (48 hours) and fed control and IUGR females. Arcuate nucleus messenger RNA ([mRNA] NPY, AgRP, and POMC) and protein expression (total and phosphorylated AMPK, Akt) was determined by quantitative reverse transcriptase–polymerase chain reaction and Western Blot, respectively. In the fed state, IUGR adult females demonstrated evidence of persistent appetite stimulation with significantly upregulated phospho (Thr¹⁷²)-AMPK α /AMPK (1.3-fold), NPY/AgRP (2.3/1.8-fold) and decreased pAkt/Akt (0.6-fold) and POMC (0.7-fold) as compared to fed controls. In controls though not IUGR adult females, fasting significantly increased pAMPK/AMPK, NPY, and AgRP and decreased pAkt/Akt and POMC. Despite obesity, fed IUGR adult females exhibit upregulated AMPK activity and appetite stimulatory factors, similar to that exhibited by fasting controls. These results suggest that an enhanced appetite drive in both fed and fasting states contributes to hyperphagia and obesity in IUGR offspring.

Keywords

appetite, AMP-activated protein kinase, intrauterine fetal growth restriction, neuropeptide Y

Introduction

Epidemiological studies have shown that adverse environmental factors during pregnancy leading to intrauterine fetal growth restriction (IUGR) and low birth weight may predispose offspring to metabolic disease development.¹⁻⁴ Anthropometric measurements, such as birth weight, represent the sum effect of genetic and epigenetic influences coupled with the intrauterine environment. Animal studies have confirmed the programming effects of low birth weight, demonstrating the predisposition to adult obesity.⁵ We have previously shown that maternal food restriction of rats during the second half of pregnancy results in IUGR newborns, which demonstrate hyperphagia, obesity, and insulin resistance in adulthood.⁶ In addition to enhanced food intake, these offspring demonstrate an impaired anorexigenic behavioral response and blunted hypothalamic signaling to exogenous leptin, a potent anorexigenic factor.⁷

Food intake and energy expenditure are tightly regulated by complex central and systemic physiological mechanisms.⁸ Appetite is primarily controlled by hypothalamic nuclei which receive input from central and peripheral neural and hormonal signals. Within the hypothalamus, the arcuate nucleus (ARC) is a key target of appetite regulatory factors and contains subsets of orexigenic (neuropeptide Y [NPY]/agouti-related protein [AgRP]) and anorexigenic (proopiomelanocortin [POMC]) neurons. Neuropeptide Y has been demonstrated to play a

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pivotal role in the control of food intake and body weight within the hypothalamus. Agouti-related protein is coexpressed with NPY, and both factors act to increase appetite and decrease metabolism and energy expenditure.⁹ Proopiomelanocortin neurons mediate anorexigenic responses by release of α -melanocyte-stimulating hormone (α -MSH). Together, these neuropeptides regulate appetite/ingestive behavior. Evidence in support of the critical role of these factors includes animal models of obesity in which levels of NPY messenger RNA (mRNA) expression are increased.^{10,11}

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a widely expressed (including neurons^{12,13}) fuel-sensing enzyme that is activated by physiological and pathological metabolic stresses which alter cellular energy status.¹⁴⁻¹⁶ Adenosine monophosphate kinase, a heterotrimer made of catalytic α subunits and regulatory β and γ subunits,¹⁶ is activated by phosphorylation of threonine 172 (Thr¹⁷²). Adenosine monophosphate kinase acts as a low-energy sensor activated by a rise in intracellular AMP/adenosine triphosphate (ATP) ratio. Following activation, AMPK phosphorylates downstream targets so that ATP-consuming pathways are inhibited and ATP-producing pathways are activated. In addition, AMPK regulates energy intake by increasing hypothalamic expression of NPY/AgRP,¹⁷ stimulating food intake and increased body weight.¹⁸ In support of this process, overexpression of a dominant negative form of AMPK in the hypothalamus decreased NPY and AgRP mRNA expression in fed rats, whereas overexpression of constitutively active AMPK augmented the fasting-induced increase in NPY and AgRP expression.¹⁷

In addition to leptin, insulin represents a potent systemic anorexigenic hormone which acts centrally within the ARC. Central insulin signaling deficiency is a cause of hyperphagia; rats with insulin-deficient diabetes have reduced ARC POMC mRNA, increased NPY mRNA, and enhanced appetite, all of which are partially attenuated by peripheral insulin therapy.¹⁹ Insulin binding to the insulin receptor induces phosphorylation of insulin receptor substrates^{20,21} which bind to the p85 regulatory subunit of phosphatidylinositol 3 kinase (PI3K). Phosphatidylinositol 3 kinase mediates the transfer of signals to downstream molecules including Akt.^{22,23} Akt is critical to the insulin signaling pathway and is required to induce glucose transport. Akt knockout mice are significantly more insulin resistant than wild-type mice.²⁴ Both AMPK and Akt act in concert to mediate ingestive behavior, with AMPK stimulating orexigenic drive and Akt stimulating anorexigenic drive.²⁵

Despite the overwhelming evidence of rapid catch-up growth in low-birth-weight humans and animals, and the current epidemic of childhood and adult obesity, the underlying mechanisms of altered hypothalamic responsiveness in these offspring are unknown. We hypothesized that hyperphagia associated with IUGR results, in part, from alteration in the balance of hypothalamic orexigenic and anorexigenic peptides. Accordingly, we examined ARC responses in adult IUGR and control offspring during both fed and fasting periods. We have previously shown that both male and female IUGR offspring are hyperphagic.^{6,7} In the present experiment,

males were sacrificed at the time of weaning for use in alternative studies unrelated to this topic.

Materials and Methods

Maternal Rat Diets

Studies were approved by the Animal Research Committee of the Los Angeles BioMedical Research Institute at Harbor–UCLA Medical Center and were in accordance with the American Association for Accreditation of Laboratory Animal Care and National Institutes of Health guidelines. The rat model utilized for maternal food restriction during pregnancy and after birth has been previously described.²⁶⁻²⁸ Briefly, the first time pregnant Sprague Dawley rats (Charles River Laboratories, Hollister, California) were housed in a facility with constant temperature and humidity and controlled 12-hour light (6 AM–6 PM)/ and dark (6 PM–6 AM) cycles. Pregnant rats were divided into 2 groups: control dams had free access to standard laboratory chow (Lab Diet 5001; Brentwood, Missouri; protein, 23%; fat, 4.5%; metabolizable energy, 3030 kcal/kg), while food restricted dams were provided 50% of the control food intake from day 10 of gestation to term (day 21) to IUGR offspring produced.

Offspring

Following birth, at day 1 of age the pups were culled to 8 per litter (4 males and 4 females) to normalize rearing. Following birth, both control and IUGR offspring were nursed (after cross-fostering the maternal food restricted pups) by ad libitum-fed dams. In the present experiment, males were sacrificed at the time of weaning for use in alternative studies unrelated to this topic. From 3 to 6 weeks of age, all females of each litter were housed together and weaned ad libitum standard laboratory chow and thereafter housed individually. For the present study only female rats, $N = 12$ per group, were used. There were a total of 6 litters per group. From each litter 2 pups were selected, 1 was fed and 1 was fasted. At 10 months of age, IUGR and control female offspring were either fasted for 48 hours beginning at 10:00 AM ($N = 6$ per group) or fed an ad libitum diet ($N = 6$ per group). All animals were sacrificed at $\sim 10:00$ AM. Offspring were decapitated under isoflurane inhalation anesthesia, brains collected, and hypothalamus dissected. Arcuate nucleus was obtained by dissecting the ventral part of the medial hypothalamus with anterior and dorsal margins (0.5 mm from the ventral surface of the medial hypothalamus) and posterior margin (border with mammillary body) as detailed in the rat brain atlas.²⁹ Subsequently, ARC tissue was snap frozen for RNA extraction and quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) or protein extraction and Western blot analysis. To confirm the hypothalamic localization of AMPK α , NPY, and α -MSH, brains were collected from 3 control females fed ad libitum diet, hypothalamus dissected, and fixed for immunohistochemistry analysis.

Table 1. Matrix Metalloproteinase Substrates

Gene (Accession Number)	Primer Sequences	
NPY (M_20373)	Forward	TATCCCTGCTCGTGTGTTTG
	Reverse	GGGCATTTTCTGTGCTTTCT
AgRP (XM_001075738)	Forward	CACGTGTGGGCCCTTTATTA
	Reverse	AGGACACAGCTCAGCAACATT
POMC (NM_139326)	Forward	AGTTCAAGAGGGAGCTGGAA
	Reverse	CTTGATGATGGCGTTCTTGA
GAPDH (NM_017008)	Forward	AGACAGCCGCATCTTCTTGT
	Reverse	CTTGCCGTGGGTAGAGTCAT

Quantitative RT-PCR

RNA extraction and complementary DNA (cDNA) synthesis were performed using TRIzol and SuperScript III reverse transcriptase (both from Invitrogen, Carlsbad, California), respectively, according to the manufacturer's protocols. Quantitative PCR (qPCR) was carried out as described previously.³⁰ The analysis was performed using the comparative cycle-threshold method,³¹ and values expressed as a fold difference from the control. The primers oligonucleotide sequences (Table 1) were obtained from Sigma-Aldrich (Woodlands, Texas).

Western Blotting

Antibodies: primary antibodies were phospho (Thr¹⁷²)-AMPK α ([pAMPK] Millipore, Lake Placid, New York), AMPK α ([AMPK] Millipore), phospho (Ser⁴⁷³)-Akt ([pAkt] Cell Signaling Technology, Inc., Danvers, Massachusetts), Akt (Cell Signaling), GAPDH (Santa Cruz Biotechnology, Inc., Santa Cruz, California). Secondary horseradish peroxidase-conjugated antibody was anti-rabbit (Bio-Rad Laboratories, Hercules, California). All commercial antibodies were optimized for binding specificity. Protein was extracted in Radio Immuno Precipitation Assay (RIPA) buffer containing protease and phosphatase inhibitors (Halt Protease Inhibitor cocktail; Thermo Scientific, Rockford, Illinois). Supernatants were obtained by microcentrifugation (12 000g; 20 minutes) and protein concentration determined by bicinchoninic acid solution (BCA) assay (Thermo Scientific). Protein expression was analyzed as previously conducted by our group.³⁰

Plasma Insulin Measurement

Blood was taken from the left ventricle of the heart just before the decapitation for the measurement of insulin levels. Blood was directly drawn into a centrifuge tube that contained no anticoagulant, promptly centrifuged (3000g for 15 minutes at 4°C), and stored at -80°C until use. Plasma insulin levels were measured by rat/mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Millipore), following the manufacturer protocol.

Immunohistochemistry

Hypothalami from control fed females (N = 3) were dissected, fixed in 4% paraformaldehyde at 4°C for 24 hours, transferred to 0.1 mol/L phosphate buffer (pH7.4) containing 30% sucrose and 0.01% sodium azide. For floating immunofluorescence staining, 50 μ m sections were cut with a vibratome (Leica, Solms, Germany). After washing, the sections were blocked for nonspecific binding with 10% donkey serum for 2 hours at room temperature. Sections were then incubated with rabbit anti-AMPK α (Epitomics, Inc, Burlingame, California), mouse anti-NPY (Santa Cruz) or sheep anti- α -MSH (Millipore) at 4°C overnight and rinsed. Sections were subsequently incubated with secondary antibody for AMPK α (anti-rabbit immunoglobulin [IgG]-Alexa488; Invitrogen), NPY (anti-mouse IgG-Alexa568; Invitrogen) or α -MSH (anti-sheep IgG-Alexa568; Invitrogen) at 4°C overnight and rinsed. Sections were processed and examined using Nikon fluorescence microscope equipped with a Nikon E600FN fluorescence microscope (Nikon, Tokyo, Japan) and photographed with HCLImage (Hamamatsu Photonics, Bridgewater, New Jersey). Every fifth section was inspected for neurons with fluorescence expression. Micrographs were processed in Image J and adjusted for brightness and contrast.

Statistical Analysis

Sample size estimates were based on a power of 80% to detect 30% changes between IUGR and control groups (assuming an expected standard deviation of 20% of mean values). This analysis results in a requirement for 6 animals in each group. Differences between groups were determined using analysis of variance (ANOVA) with Dunnett post hoc test with significance set at $P < .05$. Values are presented as the mean $\pm$ standard error of the mean (SEM).

Results

The phenotypic characteristics of this model have been previously reported.^{26,27} In this study at 1 day of age, IUGR had lower body weights as compared to control newborns (6.2 ± 0.1 vs 7.4 ± 0.1 g). However at 10 months of age, with normal nursing and post-weaning diet, IUGR were markedly heavier than control females (420 ± 16 vs 350 ± 14 g).

Orexigenic/Anorexigenic Gene Expression in ARC

Fasting versus fed state. Among control rats, fasting resulted in the expected significantly increased NPY (3.0-fold) and AgRP (2.5-fold) and decreased POMC (0.7-fold) mRNA expression as compared with the fed state. In contrast, among IUGR offspring, fasting did not alter the expression of NPY, AgRP, or POMC mRNA expression as compared with the fed state (Figure 1).

IUGR versus control. When comparing IUGR to controls, IUGR rats exhibited significantly increased NPY (2.3-fold) and AgRP (1.8-fold) and decreased POMC (0.7-fold) mRNA

Figure 1. NPY, AgRP, and POMC mRNA expression in ARC. NPY (A), AgRP (B), and POMC (C) mRNA expression under fed (■) and fasted (□) conditions in control and IUGR offspring. Control fed group was defined as 1.0-fold. *Comparisons of fed versus fasting within IUGR and control groups. #Comparisons of IUGR versus control (fed and fasting). Data are mean $\pm$ SE of N = 6 rats per treatment per group. NPY indicates neuropeptide; AgRP, agouti-related protein; POMC, proopiomelanocortin; ARC, arcuate nucleus; mRNA, messenger RNA; IUGR, intrauterine fetal growth restriction; SE, standard error.

Figure 2. AMPK and Akt activity in ARC. Protein expression (Western blot) of (A) pAMPK/AMPK and (B) pAkt/Akt under fed (■) and fasted (□) conditions in control and IUGR offspring. Control fed group was defined as 1.0-fold. *Comparisons of fed versus fasting within IUGR and control groups. #Comparisons of IUGR versus control (fed and fasting). Data are mean $\pm$ SE of N = 6 rats per treatment per group. AMPK indicates adenosine monophosphate-activated protein kinase; pAMPK, phospho (Thr¹⁷²)-AMPK α ; ARC, arcuate nucleus; IUGR, intrauterine fetal growth restriction; SE, standard error.

expression during the fed state, though no significant differences following fasting (Figure 1).

Activity of AMPK and Akt in ARC

Fasting versus fed. Among control rats, fasting induced significant increase in pAMPK/AMPK (1.7-fold) and decrease in pAkt/Akt (0.4-fold) ratios as compared with the fed state. Among IUGR rats, there were no significant changes in pAMPK/AMPK and pAkt/Akt ratios between fed and fasting states (Figure 2).

IUGR versus control. In comparison of IUGR and controls, IUGR fed rats demonstrated significantly increased pAMPK/AMPK ratio (1.3-fold) and decreased pAkt/Akt ratios (0.6-fold) as compared with control fed rats. In the fasted state, there were no significant differences in ratios between IUGR and controls (Figure 2).

Plasma Insulin Concentration

Fasting versus fed. Forty-eight hours fasting induced significant decrease in plasma insulin levels as compared to fed state in both IUGR (1.73 ± 0.05 to 0.22 ± 0.01 ng/mL) and control (1.07 ± 0.06 to 0.15 ± 0.02 ng/mL) rats.

Intrauterine fetal growth restriction versus control. IUGR exhibited a significant increase in fed state insulin levels compared with control rats (Figure 3). In the fasted state, there were no significance differences in plasma insulin levels between IUGR and controls (Figure 3).

Hypothalamic Localization of AMPK α , NPY, and α -MSH

To determine neuronal localization of AMPK α , NPY, and α -MSH (enzymatically cleaved POMC) in the ARC, immunohistochemical staining was performed in control fed rats. Neuronal AMPK α (Figure 4A and C) was localized widely in the whole hypothalamus, predominantly in ARC and the surface of choroid plexus epithelial layer lining the third ventricle. Likewise, NPY neurons (Figure 4B) were largely evident in ARC, whereas α -MSH neurons (Figure 4D) were stained broadly throughout the hypothalamus.

Discussion

Feeding behavior and energy balance are regulated in a complex manner by networks of neurons with numerous appetite-

Figure 3. Plasma insulin levels. Plasma insulin levels under fed (■) and fasted (□) conditions in control and IUGR offspring. *Comparisons of fed versus fasting within IUGR and control groups. #Comparisons of IUGR versus control (fed and fasting). Data are mean $\pm$ SE of N = 6 rats per treatment per group. IUGR indicates intrauterine fetal growth restriction; SE, standard error.

related molecules.³²⁻³⁴ We have previously demonstrated that the adult obesity exhibited by IUGR offspring is mediated, in part, via increased food intake and dysfunctional ARC anorexigenic signaling responses to exogenous leptin.²⁶ In the present study, we sought to examine whether IUGR offspring exhibit evidence of endogenous alterations in central anorexigenic signaling, including orexigenic and anorexigenic peptides. The principal findings indicate that IUGR offspring ARC signaling perceives a persistent state of “fasting,” evident by upregulated NPY and AgRP and suppressed POMC, which may result in perceived hunger despite a fed state. During the fed state, the increase in pAMPK and reduction in pAkt suggests a primary dysfunction in upstream regulatory factors which target orexigenic/anorexigenic peptides.

In accordance with fasting-augmented orexigenic drive, the present study confirmed that fasting significantly augmented NPY/AgRP and decreased POMC mRNA expression in control rats. This is likely mediated, in part, by a fasting-induced reduction in leptin and insulin and increased ghrelin, each of which act centrally at the ARC. In comparison to the control rats, IUGR rats displayed increased ARC orexigenic NPY/AgRP and decreased anorexigenic POMC mRNA expression during the fed state, indicative of a heightened “orexigenic state.” The lack of change in IUGR ARC expression between fed and fasting states indicates a persistent upregulation of orexigenic drive in programmed obese offspring. Of note, food was provided ad libitum to both adult IUGR and controls. Thus, the IUGR fed state findings are not a result of a more

rapid orexigenic response following earlier feeding but suggest a persistent state of “hunger.” Similarly, the impaired POMC expression suggests a failed “satiety” response to feeding. The increased plasma insulin in fed IUGR adults suggests that reduced central insulin is likely not responsible for the enhanced orexigenic state. Similarly, we previously demonstrated that fasted (overnight) IUGR adults have elevated plasma leptin and normal ghrelin.^{28,35} It is more likely that reduced leptin/insulin-induced ARC signaling responses contribute to upregulation of NPY/AgRP, consistent with our previous finding that the 3-week-old IUGR offspring have decreased mRNA expression of hypothalamic leptin receptor.²⁷

Both AMPK and Akt act as a molecular link between hormone/nutrient signals (eg, leptin, insulin) and cellular metabolism by ATP generating pathways.^{16,36} Hypothalamic AMPK, which is modulated by fasting and feeding, responds differently as compared to peripheral AMPK. Whereas AMPK activation in the hypothalamus promotes energy intake, pAMPK promotes energy consumption in the peripheral tissues (liver, skeletal muscle).³⁷ Accordingly, the anorexigenic hormone leptin, central insulin or MC3/4 agonists, and re-feeding³⁸ also inactivate AMPK and reduce food intake.³⁹ Conversely, orexigenic factors, including 5-aminoimidazole-4-carboxamide riboside, ghrelin,⁴⁰ and cannabinoids⁴¹ activate (phosphorylate) hypothalamic AMPK.

Akt also regulates metabolism and cell survival through its kinase activity on numerous downstream proteins in peripheral insulin-responsive tissue such as liver, skeletal muscle,^{42,43} and the central nervous system.^{43,44} PI3K-Akt inhibitors (wortmannin, LY-294002) block the inhibitory effect of central insulin injection on food intake and body weight gain in rats,^{45,46} whereas constitutive activation of ARC Akt improves insulin sensitivity.⁴⁷ In addition to leptin activation of JAK/STAT signaling, leptin activation of the classic insulin-signaling PI3K-Akt pathway could underlie many of its effects on glucose homeostasis. For instance, icv leptin quickly activates PI3K-Akt pathway.⁴⁸ Thus, leptin and insulin act synergistically to regulate Akt phosphorylation in the hypothalamus. Among IUGR females, basal fasting plasma insulin levels were extremely low and similar to control rats, whereas fed state insulin levels were significantly higher than control rats. However, there were no significant changes in pAkt/Akt ratios between fed and fasting states in IUGR rats. Thus, the continuous suppression of Akt signaling in IUGR offspring is consistent with an insulin-independent mechanism.

In accordance with the fasting-fed neuropeptide changes in control rats, there was the expected increase in pAMPK/AMPK and decrease in pAkt/Akt ratios in response to fasting. Intrauterine fetal growth restriction adults demonstrated an increased fed state level of pAMPK and reduced pAkt as compared to controls, consistent with the “orexigenic state.” There was no change in pAMPK or pAkt in response to fasting in the IUGR offspring. As both pAMPK and pAkt are upstream of NPY/AgRP and POMC, and our findings confirm the colocalization of AMPK and NPY, these results indicate a dysfunction among signaling factors which regulate neuropeptide

Figure 4. Localization of AMPK α , NPY, and α -MSH in Hypothalamus. Immunohistochemistry images of coronal hypothalamic sections stained for AMPK α (A and C; green), NPY (B; red), or α -MSH (D; red). E, Diagram showing locations of hypothalamic regions. Diagram is taken from the Paxinos and Watson rat brain atlas.²⁹ Bregma -3.24 mm. 3V indicates 3rd ventricle of the brain; MEI, medial eminence, internal layer; MEE, medial eminence, external layer; Arc, arcuate hypothalamic nucleus; ArcD, dorsal part; ArcL, lateral part; ArcM, medial part; DMV, dorsomedial hypothalamic nucleus, ventral part; dorsal part; VMH, ventromedial hypothalamic nucleus; NPY, neuropeptide; AgRP, agouti-related protein; AMPK, adenosine monophosphate-activated protein kinase; α -MSH, α -melanocyte-stimulating hormone.

expression. The continuous high levels of AMPK likely contribute to maintain higher NPY/AgRP mRNA expression and thus potentiate excessive food intake. Although we have previously shown that IUGR offspring are hyperphagic,^{6,7} measures of diurnal food intake may provide further insight as to whether the obesity consequence is a result of increased caloric ingestion during typical fed (night) states or an increased ingestion during the daylight associated fasting. The proposal of frequent small meals for weight loss programs may represent an adaptive behavioral therapy to programmed obese humans that do not have an effective satiety response to meals.

In the present study, we did not control for the effects of estrus cycle, which may somewhat modulate appetite. Rats typically have 4-day estrus cycle (proestrus, estrus, metestrus, and diestrus) with estrogen peaking during proestrus. Twenty-four-hour food intake decreases during estrous phase compared to other phases^{49,50} secondary to the inhibitory effect of estrogen on feeding. Consistent with this physiologic response, intracerebral injection of estrogen acutely decreases AMPK activity,⁵¹ while central or peripheral estrogen decreases NPY/AgRP mRNA.^{52,53} Pre-proNPY mRNA levels measured by in situ hybridization in the rat ARC during estrus were only 25% to 30% lower than other periods.⁵⁴ In the present study,

NPY mRNA levels increased in control fasting (3.0-fold) and IUGR fed (2.3-fold) states in comparison to control fed rats. Although study days were arbitrarily selected in both groups, the magnitude of the changes makes it unlikely that the findings are a result of random estrus phase differences.

In conclusion, this study demonstrates that despite obesity and ad libitum food availability, fed IUGR female adults exhibit upregulated ARC AMPK activity, reduced Akt activity, and altered mRNA expression of downstream appetite stimulatory neuropeptides, similar to that exhibited by fasting conditions in control adults. It remains uncertain whether the primary aberration in IUGR offspring is a result of altered ARC receptor expression, receptor signal coupling, or an earlier programmed dysfunction in cellular signaling. These findings provide insight into the mechanism that low birth weight can enhance offspring appetite drive, contributing to hyperphagia and obesity. When related to humans, one may expect a persistent subjective sense of hunger among programmed obese participants, regardless of the state of fed or fasting.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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