doi:10.4149/endo_2015_02_51

High plasma triglyceride levels strongly correlate with low kisspeptin in the arcuate nucleus of male rats

[1]Overgaard A, [1]Axel AMD, [2]Lie MEK, [1]Hansen HH, [1]Mikkelsen JD

[1]Neurobiology Research Unit, Copenhagen University Hospital, Rigshospitalet, Copenhagen; [2]Department of Drug Design and Pharmacology, University of Copenhagen, Denmark
E-mail: jens_mikkelsen@dadlnet.dk

Objective. It is well known that reproductive capacity is lower in obese individuals, but what mediators and signals are involved is unclear. Kisspeptin is a potent stimulator of GnRH release, and it has been suggested that kisspeptin neurons located in the arcuate nucleus transmit metabolic signals to the GnRH neurons.

Methods. In this study, we measured body weight and plasma concentrations of leptin, insulin, testosterone, and triglycerides after high fat diet exposure and correlated these parameters with the number of kisspeptin-immunoreactive neurons in the arcuate nucleus of male rats. In this model, a high fat diet (45% or 60% energy from fat, respectively) or a control diet (10% energy from fat) was provided after weaning for three months.

Results. We find a significant increase in body weight and plasma leptin concentration, but no change in the number of kisspeptin-immunoreactive cells with increased fat in the diet. Kisspeptin-immunoreactive cells are not correlated with body weight, testosterone, leptin or insulin. However, we find that the number of kisspeptin-immunoreactive cells is strongly and negatively correlated with the level of plasma triglycerides (R^2=0.49, p=0.004).

Conclusion. We find a strong negative correlation between plasma triglyceride concentrations and the number of kisspeptin neurons in the rat arcuate nucleus regardless of the percentage of fat in the diet. In line with the lipotoxicity hypothesis, our results suggest that it is the level of hypertriglyceridemia *per se* that is a detrimental factor for kisspeptin expression in the arcuate nucleus.

Key words: kisspeptin, high fat diet, triglycerides, obesity, lipotoxicity

Obesity has become a matter of great public concern due to the increased risk of co-morbidity such as metabolic dysfunctions, hypertension, and reduced fertility (Khaodhiar et al. 1999; Pasquali et al. 2007; Loret de Mola 2009). Kisspeptin, encoded by the *Kiss1* gene, is a potent stimulator of GnRH release and kisspeptin is eliciting the pre-ovulatory luteinizing hormone surge. Therefore, kisspeptin is considered essential for a proper reproductive function (Pinilla et al. 2012). Kisspeptin expression in the arcuate nucleus (ARC) is sensitive to metabolic status and the kisspeptin ARC neurons have been considered to play an important role as mediators of peripheral signals to the GnRH neurons, thereby linking obesity with reduced fertility (Castellano et al. 2010; George et al. 2010). Several studies have investigated the effect of high fat diet (HFD) exposure on kisspeptin expression in rodents, but reduced *Kiss1* mRNA and kisspeptin expression have only been shown in a mouse strain susceptible to HFD-induced fertility (Quennell et al. 2011), while wild-type mice and rats have unchanged

Corresponding author: Jens D. Mikkelsen, MD PhD., Neurobiology Research Unit, University Hospital Rigshospitalet, Bldg 6931, Juliane Maries Vej 28, 2100 Copenhagen O, Denmark; phone: +45 35456701; fax: +45 35456713; e-mail: jens_mikkelsen@dadlnet.dk.

or increased *Kiss1* mRNA expression after HFD exposure (Luque et al. 2007; Brown et al. 2008; Quennell et al. 2011; Li et al. 2012; Lie et al. 2013).

It has long been known that leptin-deficient mice are infertile and that their fertility is rescued by leptin treatment (Mounzih et al. 1997). However, the site of action for leptin in relation to fertility is still not clear. Kisspeptin neurons are proposed to express the leptin receptor (Smith et al. 2006; Backholer et al. 2010; Cravo et al. 2011; Quennell et al. 2011) and leptin has thus been suggested to be the major messenger of information on sufficient energy stores directly to kisspeptin neurons. However, recent studies fail to detect leptin receptor-dependent signaling in kisspeptin neurons (Louis et al. 2011; True et al. 2011), and while mice lacking leptin signaling have reduced *Kiss1* mRNA expression (Smith et al. 2006; Quennell et al. 2011), this phenotype is only partly rescued by leptin treatment (Smith et al. 2006). Further, transgene mice lacking the functional leptin receptor only in kisspeptin neurons do not have compromised puberty or fertility (Donato et al. 2011). Hence, the effect of leptin on fertility is likely to be, at least partly, independent of kisspeptin signaling.

It is well known that co-morbidities are linked to obesity; however significant variation exists between individuals, with some severely obese showing no signs of metabolic dysfunctions and *vice versa* (Karelis et al. 2004; Capeau et al. 2005). A hypothesis explaining this variation is the hypothesis of lipotoxicity, which states that it is not the volume of adipose tissue, but rather the capability of the body to clear lipids from the circulation, which determines the adverse effects of adiposity (Sorensen et al. 2010). When the fat storage capacity in the adipose tissue is reached, fat is directed to non-adipose tissue, where storage capacity is very limited. Lipids are therefore degraded through non-oxidative pathways resulting in toxic reactive lipids and ultimately apoptosis of cells in non-adipose tissue (Sorensen et al. 2010). Hence, the lipotoxicity hypothesis explains the concomitant deterioration of pancreas, liver, kidney, skeletal muscle, blood vessels, and heart observed in patients with the metabolic syndrome.

Lipotoxicity has also been reported in the hypothalamus (De Souza et al. 2005; Milanski et al. 2009; Moraes et al. 2009). Specifically, a long-term HFD exposure has been shown to increase inflammatory markers such as TNFα, pJNK, IL-1β, and IL-6 in the hypothalamus of male rats (De Souza et al. 2005; Milanski et al. 2009; Moraes et al. 2009). An increase in neuronal apoptosis and reduction of synaptic inputs specifically in the ARC after HFD exposure has also been reported (Moraes et al. 2009). In the same line, pJNK-mediated endoplasmic reticulum (ER) stress after exposure to a non-esterified fatty acid in a hypothalamic neuronal cell line has been reported (Mayer and Belsham 2010).

Moreover, obesity models in rodents have varying efficacy on co-morbidity parameters of obesity and even on bodyweight, depending on species, genetic background, and diet (Nilsson et al. 2012; Rosini et al. 2012). Further, even within a single rat strain, such as Sprague-Dawley, there is variation in the sensitivity towards becoming obese on a specific high caloric diet (Levin and Keesey 1998). This variation in the susceptibility to experiencing adverse effects of HFD, suggests that it is not the diet *per se*, which induces the co-morbidity of obesity. In the same line, previous studies do not find an adverse effect of HFD on kisspeptin expression (Luque et al. 2007; Brown et al. 2008; Quennell et al. 2011; Li et al. 2012; Lie et al. 2013). We therefore hypothesize that a metabolic marker, rather than HFD *per se*, is important for kisspeptin expression.

Materials and Methods

Animals. Male Sprague-Dawley rats were obtained from Taconic Inc., Denmark, and kept under constant conditions of light (12 h of light from 2:00 pm), temperature (18-22°C), and relative air humidity (55±10%). Upon arrival the rats (n=6 per group; age 24 days) were randomly assigned into three groups with free access to tap water and either standard chow (Altromin 1324 with 10% kcal from fat and energy density of 2.85 kcal/g, Altromin GmbH, Germany) or a HFD with either 45% (#D12451; energy density 4.73 kcal/g, Research Diets, USA) or 60% kcal from fat (#D12492; energy density 5.24 kcal/g, Research Diets, USA). After 12 weeks on their respective diets, tail vein blood was collected in heparinised tubes and animals were anaesthetized with Mebumal and perfused transcardially with 0.9% saline for 5 min, followed by 4% paraformaldehyde-phosphate buffer for 10 min (0.1 M; pH 7.4). The brains were rapidly isolated and postfixated in the same fixative overnight and thereafter kept in 0.05 M phosphate-buffered saline (PBS) at 4°C.

The experiment has been approved (J.no. 2005/561-1055) and conducted in accordance with the guidelines of the Animal Experimentation Inspectorate, Ministry of Justice, Denmark.

Immunocytochemistry. The fixed brains were soaked with 30% sucrose-PBS solution for two days,

and thereafter cut into 4 series of 40 μm free-floating coronal sections through the ARC. The sections were evaluated for kisspeptin-immunoreactivity, as previously described (Bentsen et al. 2010). In brief, one series of sections were washed in PBS, incubated with 1% H_2O_2 in PBS for 10 min to block endogen peroxidase, followed by 20 min in PBS containing 0.3% Triton X-100, 5% swine serum, and 1% bovine serum albumin (BSA), to block non-specific binding. The sections were then incubated in the primary antiserum JLV-1 diluted 1:200 in 0.3% Triton-X 100 and 1% BSA and gently shaken overnight at 4°C. This primary antiserum raised against N-terminal kisspeptin-52 has been shown not to cross-react with related arginine-phenylalanine (RF)-amides (Desroziers et al. 2010; Overgaard et al. 2013). After washing in PBS containing 0.1% Triton X-100 (T-PBS), the sections was incubated for 1 h in biotinylated donkey anti-rabbit antiserum (Jackson Labs, 711-066-152) diluted 1:1000 in T-PBS with 0.3% BSA. After another T-PBS wash, the sections were incubated with 0.4% avidin-biotin-peroxidase complex (Vector Elite Kit™, Vector Labs, USA) diluted in T-PBS. They were developed in 0.05% diaminobenzidine (Sigma-Aldrich, USA) with 0.05% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6, 5°C) for 10 min. The sections were mounted and cover slipped in Pertex® (HistoLab, Sweden). The total number of kisspeptin-immunoreactive (-ir) cells in the entire ARC in one series of sections was quantified for each rat under bright field illumination (Zeiss Imager Z.1 microscope) by the same person, and the identity of the rats was blinded through the immunocytochemical procedure and during quantification.

Plasma analysis. Plasma insulin was measured using a standard sandwich ELISA assay (10-1250-01, Mercodia AB, Sweden), total triglycerides were measured using a biochemical assay (TR0100, Sigma-Aldrich, Denmark), and free testosterone was measured using a coat-a-count RIA kit based on 125I labeled testosterone (TKTF1; Siemens Medical Solutions, USA), following the instructions of the manufacturer of the respective assays. Plasma leptin was determined with a Rat Leptin Elisa kit (90040, Crystal Chem Inc, USA) using a standard sandwich technique. The instructions of the manufacturer were followed, except from the background absorbance measurement, which was measured at 655nm absorbance. Limited plasma volume was available, and therefore not all animals were included in all plasma analyses. See Table 1 for the number of animals in the different groups analyzed for each analysis.

Statistical analysis. Differences between the three diet groups were analyzed using a one-way ANOVA followed by Tukey's multiple comparisons test, except for analysis of body weight, where the groups showed significant different standard deviations ($p<0.05$, Brown-Forsythe test). Therefore, a Kruskal-Wallis test was applied to analyze differences in body weight between the three diet groups. Data are presented as mean ± standard error of mean (SEM) in Fig. 1A and Table 1. Pearson's correlations were used in all correlations, and for all correlations, the three diet groups were pooled. The p-value and R^2 of the correlations are presented in Fig. 1B and Table 2. For all analyses $p<0.05$ was considered statistically significant.

Results

High fat diet and metabolic markers. Male rats were exposed to HFD after weaning and after three months on the 45% HFD or the 60% HFD body weights were significantly increased ($p<0.001$, Table 1). In addition, exposure to 60% HFD caused a significant increase in plasma leptin levels ($p<0.05$, Table 1). It is noteworthy that both HFD groups had significantly increased variability in body weight compared to the control group

Table 1
Body weight and plasma concentrations of metabolic markers

Fat in diet (% kcal)	10%	45%	60%
Body weight (g)	405.8±3.0 (6)	**505.2±25.4**[a] (6)	**547.7±15.7**[a] (6)
Leptin (ng/ml)	4.34±1.37 (2)	10.90±1,79 (4)	**19.43±3.93***(4)
Insulin (ng/ml)	0.45±0.03 (2)	1.05±0.26 (6)	1.81±0.69 (4)
Triglycerides (mg/ml)	0.93±0.20 (4)	1.22±0.11 (6)	0.99±0.11 (5)
Testosterone (pg/ml)	5.30±2.40 (2)	1.86±0.19 (2)	7.39±5.06 (3)

Data represent group means ± SEM.
Numbers in parentheses are the n values.
[a] High fat diet significantly affects body weight ($p<0.001$, Kruskal-Wallis test).

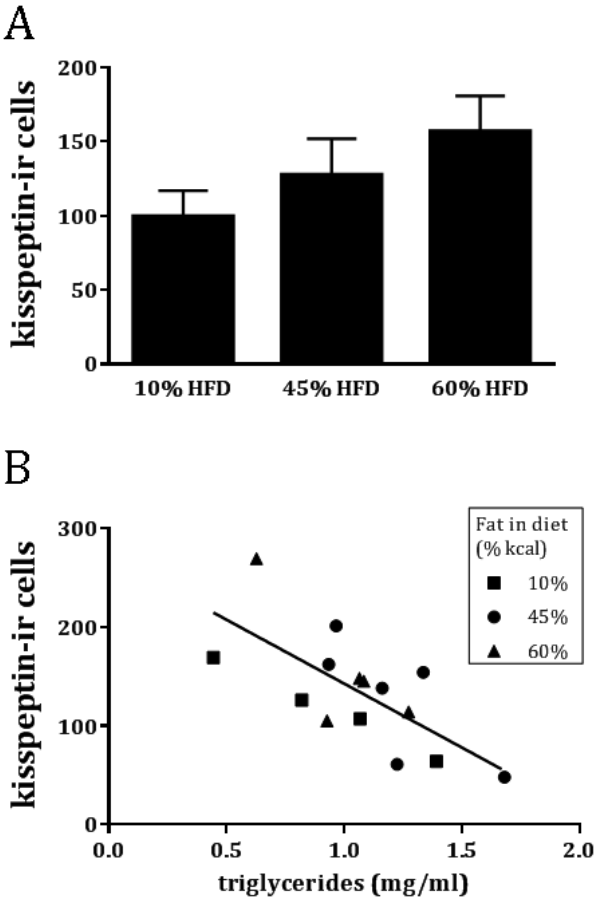

Fig. 1. The number of kisspeptin-immunoreactive (-ir) cells in the arcuate nucleus (ARC) in relation to diet and total plasma triglycerides. A) The number of kisspeptin-ir cells in the ARC of male rats on a diet with 45% and 60% energy from fat is not different from controls (p=0.22, one-way ANOVA). B) Plasma triglyceride concentrations are negatively correlated with kisspeptin-ir cells in the ARC (p=0.004, R^2=0.49). HFD - high fat diet.

(p<0.05), indicating different susceptibility to body weight gain on the given diets.

The other metabolic markers including insulin, triglyceride, and testosterone plasma levels did not differ between the diet groups and were not affected by the HFD *per se* (Table 1). However, both insulin (p<0.05) and leptin (p<0.001) plasma concentrations positively correlated with body weight (Table 2). On the contrary, plasma concentrations of triglyceride (p=0.91) and testosterone (p=0.74) did not correlate with the body weight (Table 2).

Triglyceride levels correlate to kisspeptin irrespective of diet. To relate the metabolic markers to kisspeptin expression, immunocytochemistry was performed in a series of sections covering the entire ARC. The number of kisspeptin-ir cells in the ARC did not differ between the three diet groups (p=0.22; Fig. 1A), and neither body weight nor plasma concentrations of leptin, insulin, or testosterone correlated with the number of kisspeptin-ir cells in the ARC (Table 2).

We find a significant negative correlation between plasma concentration of total triglycerides and the number of kisspeptin-ir cells in the ARC (p<0.004, R^2=0.49; Fig. 1B and Table 2). When the diet groups were analyzed separately, significant correlation was only found in the control group (controls, R^2=0.99, p=0.004; 45% HFD, R^2=0.58, p=0.078; 60%HFD, R^2=0.66, p=0.095).

Discussion

Previous studies investigating the effect of HFD on *Kiss1* mRNA expression have reported only plasma leptin and not triglyceride levels and no correlations have been done (Quennell et al. 2011; Li et al. 2012). Thus, our study is the first to show that kisspeptin expression correlates with plasma triglyceride levels and not with leptin levels or body weight. Intriguingly, this correlation is evident also within the control groups, emphasizing the relative importance of plasma triglyceride levels compared to other obesity parameters. This finding is in line with the lipotoxicity hypothesis, stating that while triglycerides stored in adipose tissue are physiologically inert, circulating triglycerides can be detrimental (Sorensen et al. 2010). Moreover, HFD has been shown to cause cellular stress in the hypothalamus leading to increased hypothalamic apoptosis and central leptin and insulin resistance (De Souza et al. 2005; Moraes et al. 2009). Further, it has been demonstrated *in vitro* that fatty acid exposure causes ER stress in a hypothalamic neuronal cell model, supporting the importance of triglyceridemia in the pathophysiology of obesity, also at targets within the hypothalamus (Mayer and Belsham 2010). While acute intracerebroventricular injection of fatty acids inhibits food intake and glucose production (Obici et al. 2002), further studies with prolonged fatty acid injection and subsequent localization of inflammatory markers in kisspeptin neurons, will be needed to determine whether it is indeed the fatty acids that reduce kisspeptin expression directly in the hypothalamus and whether this is accompanied by inflammation and apoptosis. Therefore, we conclude that elevated plasma triglyceride levels itself or another mechanism induced by this biomarker may cause a down-regulation of kisspeptin expression in the ARC.

Table 2
Correlation of kisspeptin, body weight and metabolic plasma markers

Correlation with body weight	**p-value**	**R^2**	**n**
Leptin (ng/ml)	**0.001**	**0.85**	10
Insulin (ng/ml)	**0.017**	**0.53**	12
Triglycerides (mg/ml)	0.91	0.001	15
Testosterone (pg/ml)	0.74	0.025	7
Correlation with kisspeptin	**p-value**	**R^2**	**n**
Body weight (g)	0.14	0.14	17
Leptin (ng/ml)	0.26	0.17	10
Insulin (ng/ml)	0.11	0.26	12
Triglycerides (mg/ml)	**0.004**	**0.49**	15
Testosterone (pg/ml)	0.15	0.37	7

Correlations based on data from all three diet groups.
Kisspeptin is the number of immunoreactive cells in ARC.
Leptin, insulin, triglycerides and testosterone are plasma concentrations.

We find that the number of kisspeptin-ir cells after HFD exposure is unchanged compared to control diet, which is in line with previous reports, showing no change or increased levels of *Kiss1* mRNA or kisspeptin expression in obese rodents (Luque et al. 2007; Brown et al. 2008; Castellano et al. 2011; Quennell et al. 2011; Li et al. 2012; Lie et al. 2013). Decreased testosterone levels are reported in obese men and mice (Luque et al. 2007; Corona et al. 2013). Because kisspeptin expression in the ARC is inhibited by sex steroids (Smith et al. 2005), the tendency to increased kisspeptin in the HFD groups could be explained by decreased testosterone levels.

There is some controversy whether leptin is an important regulator of *Kiss1* mRNA expression (Castellano et al. 2006; Smith et al. 2006; Luque et al. 2007; Donato et al. 2011; Quennell et al. 2011). However, we propose that while leptin could be important as a permissive signal during energy deficit, leptin is not the key signal for the detrimental metabolic effects of obesity to kisspeptin neurons. Likewise, we found no correlation between plasma insulin levels and the number of kisspeptin-ir cells. Inactivation of the insulin receptor specifically in the brain gives rise to an infertile and metabolic disturbed mouse phenotype, suggesting an important central role of insulin for fertility (Bruning et al. 2000). However, the central actions of insulin is not likely to be mediated by kisspeptin, since reduced *Kiss1* mRNA expression in diabetic mice is not rescued by insulin administration (Castellano et al. 2006), and *in vitro* studies confirm the lack of effect of insulin on *Kiss1* mRNA expression (Luque et al. 2007).

In conclusion, we find a strong negative correlation between plasma triglyceride concentrations and the number of kisspeptin neurons in the rat ARC regardless of the percentage of fat in the diet. In line with the lipotoxicity hypothesis, our results suggest that it is the level of hypertriglyceridemia *per se* that is a detrimental factor for kisspeptin expression in the ARC.

Acknowledgements

This study was supported by The Danish Medical Research Council and the NOVO Nordisk Foundation.

References

Backholer K, Smith JT, Rao A, Pereira A, Iqbal J, Ogawa S, Li Q, Clarke IJ: Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology 151, 2233-2243, 2010. http://dx.doi.org/10.1210/en.2009-1190

Bentsen AH, Ansel L, Simonneaux V, Tena-Sempere M, Juul A, Mikkelsen JD: Maturation of kisspeptinergic neurons coincides with puberty onset in male rats. Peptides 31, 275-283, 2010. http://dx.doi.org/10.1016/j.peptides.2009.11.017

Brown RE, Imran SA, Ur E, Wilkinson M: KiSS-1 mRNA in adipose tissue is regulated by sex hormones and food intake. Mol Cell Endocrinol 281, 64-72, 2008. http://dx.doi.org/10.1016/j.mce.2007.10.011

Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. Science 289, 2122-2125, 2000. http://dx.doi.org/10.1126/science.289.5487.2122

Capeau J, Magre J, Lascols O, Caron M, Bereziat V, Vigouroux C, Bastard JP: Diseases of adipose tissue: genetic and acquired lipodystrophies. Biochem Soc Trans 33, 1073-1077, 2005. http://dx.doi.org/10.1042/BST20051073

Castellano JM, Navarro VM, Fernandez-Fernandez R, Roa J, Vigo E, Pineda R, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M: Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. Diabetes 55, 2602-2610, 2006. http://dx.doi.org/10.2337/db05-1584

Castellano JM, Bentsen AH, Mikkelsen JD, Tena-Sempere M: Kisspeptins: Bridging energy homeostasis and reproduction. Brain Res 1364, 129-138, 2010. http://dx.doi.org/10.1016/j.brainres.2010.08.057

Castellano JM, Bentsen AH, Sanchez-Garrido MA, Ruiz-Pino F, Romero M, Garcia-Galiano D, Aguilar E, Pinilla L, Dieguez C, Mikkelsen JD, Tena-Sempere M: Early metabolic programming of puberty onset: impact of changes in postnatal feeding and rearing conditions on the timing of puberty and development of the hypothalamic kisspeptin system. Endocrinology 152, 3396-3408, 2011. http://dx.doi.org/10.1210/en.2010-1415

Corona G, Rastrelli G, Monami M, Saad F, Luconi M, Lucchese M, Facchiano E, Sforza A, Forti G, Mannucci E, Maggi M: Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. Eur J Endocrinol 168, 829-843, 2013. http://dx.doi.org/10.1530/EJE-12-0955

Cravo RM, Margatho LO, Osborne-Lawrence S, Donato J Jr., Atkin S, Bookout AL, Rovinsky S, Frazao R, Lee CE, Gautron L, Zigman JM, Elias CF: Characterization of Kiss1 neurons using transgenic mouse models. Neuroscience 173, 37-56, 2011. http://dx.doi.org/10.1016/j.neuroscience.2010.11.022

De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA: Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146, 4192-4199, 2005. http://dx.doi.org/10.1210/en.2004-1520

Desroziers E, Mikkelsen J, Simonneaux V, Keller M, Tillet Y, Caraty A, Franceschini I: Mapping of kisspeptin fibres in the brain of the pro-oestrous rat. J Neuroendocrinol 22, 1101-1112, 2010. http://dx.doi.org/10.1111/j.1365-2826.2010.02053.x

Donato J Jr., Cravo RM, Frazao R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S Jr., Coppari R, Zigman JM, Elmquist JK, Elias CF: Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. J Clin Invest 121, 355-368, 2011. http://dx.doi.org/10.1172/JCI45106

George JT, Millar RP, Anderson RA: Hypothesis: kisspeptin mediates male hypogonadism in obesity and type 2 diabetes. Neuroendocrinology 91, 302-307, 2010. http://dx.doi.org/10.1159/000299767

Karelis AD, St-Pierre DH, Conus F, Rabasa-Lhoret R, Poehlman ET: Metabolic and body composition factors in subgroups of obesity: what do we know? J Clin Endocrinol Metab 89, 2569-2575, 2004. http://dx.doi.org/10.1210/jc.2004-0165

Khaodhiar L, McCowen KC, Blackburn GL: Obesity and its comorbid conditions. Clin Cornerstone 2, 17-31, 1999. http://dx.doi.org/10.1016/S1098-3597(99)90002-9

Levin BE, Keesey RE: Defense of differing body weight set points in diet-induced obese and resistant rats. Am J Physiol 274, R412-419, 1998.

Li XF, Lin YS, Kinsey-Jones JS, O'Byrne KT: High-fat diet increases LH pulse frequency and kisspeptin-neurokinin B expression in puberty-advanced female rats. Endocrinology 153, 4422-4431, 2012. http://dx.doi.org/10.1210/en.2012-1223

Lie MEK, Overgaard A, Mikkelsen J: Effects of a postnatal high-fat diet exposure on puberty onset, the estrous cycle, and kisspeptin in female rats. Reprod Biol 13, 298-308, 2013. http://dx.doi.org/10.1016/j.repbio.2013.08.001

Loret de Mola JR: Obesity and its relationship to infertility in men and women. Obstet Gynecol Clin North Am 36, 333-346, 2009. http://dx.doi.org/10.1016/j.ogc.2009.03.002

Louis GW, Greenwald-Yarnell M, Phillips R, Coolen LM, Lehman MN, Myers MG Jr: Molecular mapping of the neural pathways linking leptin to the neuroendocrine reproductive axis. Endocrinology 152, 2302-2310, 2011. http://dx.doi.org/10.1210/en.2011-0096

Luque RM, Kineman RD, Tena-Sempere M: Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: analyses using mouse models and a cell line. Endocrinology 148, 4601-4611, 2007. http://dx.doi.org/10.1210/en.2007-0500

Mayer CM, Belsham DD: Palmitate attenuates insulin signaling and induces endoplasmic reticulum stress and apoptosis in hypothalamic neurons: rescue of resistance and apoptosis through adenosine 5' monophosphate-activated protein kinase activation. Endocrinology 151, 576-585, 2010. http://dx.doi.org/10.1210/en.2009-1122

Milanski M, Degasperi G, Coope A, Morari J, Denis R, Cintra DE, Tsukumo DM, Anhe G, Amaral ME, Takahashi HK, Curi R, Oliveira HC, Carvalheira JB, Bordin S, Saad MJ, Velloso LA: Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci 29, 359-370, 2009. http://dx.doi.org/10.1523/JNEUROSCI.2760-08.2009

Moraes JC, Coope A, Morari J, Cintra DE, Roman EA, Pauli JR, Romanatto T, Carvalheira JB, Oliveira AL, Saad MJ, Velloso LA: High-fat diet induces apoptosis of hypothalamic neurons. PLoS One 4, e5045, 2009. http://dx.doi.org/10.1371/journal.pone.0005045

Mounzih K, Lu R, Chehab FF: Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology 138, 1190-1193, 1997. http://dx.doi.org/10.1210/en.138.3.1190

Nilsson C, Raun K, Yan FF, Larsen MO, Tang-Christensen M: Laboratory animals as surrogate models of human obesity. Acta Pharmacol Sin 33, 173-181, 2012. http://dx.doi.org/10.1038/aps.2011.203

Obici S, Feng Z, Morgan K, Stein D, Karkanias G, Rossetti L: Central administration of oleic acid inhibits glucose production and food intake. Diabetes 51, 271-275, 2002. http://dx.doi.org/10.2337/diabetes.51.2.271

Overgaard A, Tena-Sempere M, Franceschini I, Desroziers E, Simonneaux V, Mikkelsen JD: Comparative analysis of kisspeptin-immunoreactivity reveals genuine differences in the hypothalamic Kiss1 systems between rats and mice. Peptides 45, 85-90, 2013. http://dx.doi.org/10.1016/j.peptides.2013.04.013

Pasquali R, Patton L, Gambineri A: Obesity and infertility. Curr Opin Endocrinol Diabetes Obes 14, 482-487, 2007. http://dx.doi.org/10.1097/MED.0b013e3282f1d6cb

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M: Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 92, 1235-1316, 2012. http://dx.doi.org/10.1152/physrev.00037.2010

Quennell JH, Howell CS, Roa J, Augustine RA, Grattan DR, Anderson GM: Leptin deficiency and diet-induced obesity reduce hypothalamic kisspeptin expression in mice. Endocrinology 152, 1541-1550, 2011. http://dx.doi.org/10.1210/en.2010-1100

Rosini TC, Silva AS, Moraes C: Diet-induced obesity: rodent model for the study of obesity-related disorders. Rev Assoc Med Bras 58, 383-387, 2012. http://dx.doi.org/10.1016/S0104-4230(12)70211-7

Smith JT, Dungan HM, Stoll EA, Gottsch ML, Braun RE, Eacker SM, Clifton DK, Steiner RA: Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. Endocrinology 146, 2976-2984, 2005. http://dx.doi.org/10.1210/en.2005-0323

Smith JT, Acohido BV, Clifton DK, Steiner RA: KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. J Neuroendocrinol 18, 298-303, 2006. http://dx.doi.org/10.1111/j.1365-2826.2006.01417.x

Sorensen TI, Virtue S, Vidal-Puig A: Obesity as a clinical and public health problem: is there a need for a new definition based on lipotoxicity effects? Biochim Biophys Acta 1801, 400-404, 2010.

True C, Kirigiti MA, Kievit P, Grove KL, Smith MS: Leptin is not the critical signal for kisspeptin or luteinising hormone restoration during exit from negative energy balance. J Neuroendocrinol 23, 1099-1112, 2011. http://dx.doi.org/10.1111/j.1365-2826.2011.02144.x