HHS Public Access

Author manuscript

Obesity (Silver Spring). Author manuscript; available in PMC 2017 August 03.

Published in final edited form as:

Obesity (Silver Spring). 2017 March ; 25(3): 553–560. doi:10.1002/oby.21754.

Acute Recapitulation of the Hyperinsulinemia and Hyperlipidemia characteristic of Metabolic Syndrome suppresses Gonadotropins

Justin Chosich[1], **Andrew P. Bradford**[1], **Amanda A Allshouse**[4], **Jane E. B. Reusch**[2,3], **Nanette Santoro**[1,*], and **Irene E Schauer**[2,3,*]

[1] Department of Obstetrics and Gynecology, University of Colorado School of Medicine

[2] Department of Medicine, Division of Endocrinology, Metabolism, and Diabetes, University of Colorado School of Medicine

[3] Endocrinology Section, Denver Veterans Affairs Medical Center

[4] Department of Biostatistics and Informatics, Colorado School of Public Health

Abstract

Objective—To determine the effect of lipid/heparin versus saline infusion, with or without concurrent euglycemic hyperinsulinemia on serum FSH and LH. Obesity is associated with hyperlipidemia, insulin resistance, and relative hypogonadotropic hypogonadism. We hypothesized that acutely elevated fatty acids and insulin would impair gonadotropin secretion.

Methods—Regularly cycling women and men who were non-obese underwent a crossover 6-hour infusion study over 4 visits. Participants received infusions of: saline-control, lipid/heparin, insulin and lipid/heparin plus insulin. Serum FSH and LH were measured by immunoassay.

Results—In women (n=10), infusion of lipid plus insulin significantly reduced LH, from 4.6 (3.7-5.4) [mean (95% confidence interval)] to 3.3 (2.3-4.4); p=0.03 and FSH from 3.9 (3.2-4.6) to 3.1, (2.3-3.8) IU/L; p=0.03 compared to saline-control. Similarly, in men (n=10), LH, 3.3 (2.4-4.1) IU/L and FSH, 2.1 (1.4-2.8) IU/L were significantly reduced after the combined infusion, (2.2 (1.3-3.1) IU/L and 1.5 (0.8-2.1) IU/L; p=0.03, p=0.02, respectively). Neither lipid nor insulin alone significantly impacted gonadotropin levels compared to saline-control.

Conclusions—Hyperinsulinemia combined with elevated lipids acutely suppresses LH and FSH, providing a possible mechanism underlying the relative hypogonadotropic hypogonadism of obesity. Effects of insulin on the hypothalamic-pituitary-gonadal axis may be dependent on the concomitant metabolic environment.

Corresponding author: Nanette Santoro, MD, Department of Obstetrics and Gynecology, University of Colorado School of Medicine, Campus Box B-198, 12631 E. 17th Ave., Aurora, CO 80045, USA, Phone: 303 724 2041, Fax: 303 724 3512, nanette.santoro@ucdenver.edu.

*co-Senior Authors

Disclosure: The authors declare no conflict of interest

Keywords

Metabolic Syndrome; hypothalamic-pituitary-gonadal axis; FSH; LH

Introduction

Obesity is associated with high circulating free fatty acids and triglycerides (TG), insulin resistance, and impaired secretion of luteinizing (LH) and follicle-stimulating hormone (FSH). Obesity can lead to metabolic syndrome, in which, an individual has a clustering of three or more of the following: hypertension, low HDL, hypertriglyceridemia, high fasting glucose, and abdominal obesity. Collectively, obesity has been implicated in reproductive dysfunction and adverse pregnancy outcomes (1, 2, 3, 4). Thus it is important to understand the mechanisms by which obesity impacts the reproductive axis.

We have previously shown that pituitary LH and FSH secretion is impaired in women with obesity (5). Subjects with obesity had a reduction in mean serum LH and FSH, and LH pulse amplitude, compared to women of normal weight included as study controls, and a blunted pituitary response to exogenous gonadotropin releasing hormone (GnRH) (6). These data imply that metabolic changes due to obesity result in a functional impairment of the hypothalamic-pituitary-ovarian axis at the level of the pituitary, impacting LH and FSH synthesis and/or secretion (6, 7, 8, 9, 10, 11).

Numerous studies have implicated insulin and free fatty acids in the regulation of LH and FSH synthesis and secretion. However, results have often been contradictory and interpretations frequently are made in the context of polycystic ovarian syndrome (PCOS) and/or frank type 2 diabetes. Elevated insulin levels have been shown to suppress LH in sheep (12) but appeared to have no effect in rhesus monkeys (13). It is, however, difficult to separate effects of insulin from those of hypoglycemia in animal models in which insulin is infused. In hyperinsulinemic, euglycemic clamp (HEC) studies of women, circulating LH levels are slightly reduced in women undergoing insulin infusion, as well as those with worsening insulin resistance (14). In vitro studies are no more consistent. Insulin was found to stimulate LH and FSH release from cultured pituitary cells(15), while free fatty acid administration induced LHβ mRNA but suppressed that of FSHβ (16). The latter study did not examine hormone release. To date, studies have not addressed the combinatorial effects of both insulin and triglycerides (TG)s/free fatty acids on gonadotropins under euglycemic conditions in metabolically healthy human subjects.

We hypothesized that the reproductive milieu of women with obesity includes both insulin resistance and dyslipidemia, and that both factors together might be responsible for the pituitary impairment we have observed in such women. We further hypothesized that if our notion were correct, we should be able to reproduce a similar reproductive defect in individuals characterized as healthy and non-obese by induction of insulin resistance and hyperlipidemia. To test this hypothesis, we investigated serum LH and FSH profiles in women and men of non-obese weight, during a lipid or saline infusion in the presence or absence of hyperinsulinemia with euglycemia using a hyperinsulinemic, euglycemic clamp (HEC). We measured LH and FSH across the infusions to determine whether a short-term

mimicking of the metabolic changes of interest (elevated lipids and hyperinsulinemia) impacted the hypothalamic-pituitary-gonadal axis, inducing the impaired gonadotropin secretion that is characteristically observed in obesity.

Methods

Participants

This study was performed as a secondary analysis of an ongoing study. Parent study recruitment consisted of non-obese (BMI 18-28kg/m^2) men and women in good health, aged 18-40 years old,), and reporting sedentary to moderately active lifestyles (vigorous exercise no more than 3 times per week). History, physical exam and laboratory testing confirmed good health status, defined as absence of exclusionary comorbid conditions including hypertension, pregnancy, impaired glucose tolerance (75g oral glucose tolerance test with 2hour glucose ≥140mg/dl), impaired fasting glucose (≥100mg/dl), overt diabetes, TG >250mg/dl, liver or kidney disease, pulmonary disease, chronic inflammatory conditions, coagulopathy, anemia, abnormal cardiac function or evidence of ischemic heart disease. Additional exclusions included: use of medications known to impact insulin production or sensitivity, the presence of soy or egg allergies (due to possible reaction to the lipid infusate), any type of tobacco use, and use of any medications or supplements that would impact reproductive hormones, including systemic hormonal contraception. All women were premenopausal, with a history of regular menstrual cycles, and underwent study only during the follicular phase of the menstrual cycle. Male participants were without reproductive complaints and not taking reproductive medications. Detailed sexual function information and genital examinations were not performed. All subjects refrained from any exercise for three days prior to study visits.

Study Design

This was a secondary analysis of a subset of samples from a parent study designed to examine the impact of acute fatty acid elevation and the resulting insulin resistance on exercise performance parameters in adults characterized as healthy and non-obese (17, 18). Participants underwent a maximum of five study visits; a screening visit with a DEXA scan to determine body composition, and a series of 4 visits, in random order, for infusion of saline-control or lipid/heparin, each in the presence or absence of a HEC (Figure 1). Heparin was co-administered with lipid infusion to enhance liberation of free fatty acids. Visits were at least one week apart and for women, restricted to days 5-10 of the follicular phase of the menstrual cycle to control for potential confounding effects of the menstrual cycle on glucose metabolism and insulin resistance (19). Randomization of visit order was performed by Clinical Translational Research Center (CTRC) bioinformatics personnel using a CTRC randomization program and stratified by gender. All study visits were preceded by three days of a prescribed and provided diet (50% carbohydrate, 30% fat, 20% protein) during which time participants were required to abstain from moderate to vigorous exercise. Subjects were provided with breakfast at the time of infusion start. Breakfast composition was adjusted for the saline and lipid infusion visits such that cumulative morning caloric intake was constant across all visits. Blood samples, for measurement of hormones, were obtained at regular intervals, beginning immediately prior to the start of each 6-hour infusion

(Figure 1). Not all participants completed the full protocol. The study was approved, by the University of Colorado Institutional Review Board, and informed consent was obtained from all participants.

Lipid/heparin Infusion

Participants underwent saline-control or lipid/heparin infusions (Liposyn II or Intralipid; 20% lipid emulsion at 45 cc/hour, heparin at 0.4 U/kg/min), in the presence or absence of a HEC, conducted at 4 separate visits, for 6 hours commencing at approximately 6AM. The study was initiated with Liposyn II (Abbott Laboratories, North Chicago, IL; 10% safflower oil, 10% soybean oil, 1.2% egg phosphatides and 2.5% glycerin; major component fatty acids: approximately 65.8% linoleic, 17.7% oleic, 8.8% palmitic, 3.4% stearic, and 4.2% linolenic acid), but due to a product recall, some participants received Intralipid (Baxter Healthcare Corporation, Deerfield, IL; 20% Soybean oil, 1.2%egg yolk phospholipids, 2.25% glycerin; major component fatty acids: linoleic (44-62%), oleic (19-30%), palmitic (7-14%), linolenic (4-11%) and stearic (1.4-5.5%) instead. Liposyn and Intralipid are reported to induce similar degrees of insulin resistance (20, 21) and this was confirmed within this study (see results).

Hyperinsulinemic euglycemic clamp (HEC) studies

HECs were performed during the final 3 hours of a 6- hour saline or lipid/heparin infusion in the late morning. Clamp visits included a single stage (40 mU/m^2/min) insulin infusion performed as previously reported (22, 23). Insulin sensitivity is reported as glucose infusion rate (average space-corrected glucose infusion rate in mg glucose/kg lean body mass/min over the last 30 minutes).

Caloric Estimates

Daily caloric needs for the prescribed diet were determined from fat free mass [FFM; determined by Dual Energy X-ray Absorptiometry (DEXA), as described previously (24)] and activity using the equation: “Total Energy = ([FFM X 23.9] + 372) X activity factor” where FFM = lean mass + bone mineral content in kg. An activity factor of 1.4-1.6 was used for this largely sedentary population (25).

Hormone assays

Serum FSH and LH were measured using specific, solid-phase immunofluorometric assays (DELFIA, Perkin Elmer, Turku, Finland) as previously described (24, 26). Inter-assay and intra-assay coefficients of variation (CV) were 6.3% and 4.2%, respectively for FSH, and 4.7% and 5.4% for LH. Estradiol and sex hormone binding globulin (SHBG) were measured by immunoassay (ADVIA Centaur XP, Siemens, Malvern, PA). Inter-assay and intra-assay CVs were: 10.6% and 10.6% for estradiol. SHBG was measured using a single kit, therefore there is no inter-assay CV to report. Intra-assay CV for SHBG was 3%. Testosterone was measured by Access Testosterone assay (Beckman Coulter, Brea, CA); intra- and inter-assay CVs were 2.1% and 5.1%, respectively. .

Metabolic Assays

Serum insulin, Hemoglobin (Hb) A1C, Glucose, non-esterified fatty acids (NEFA) and TGs, were determined, by the University of Colorado CTRC laboratory, as described previously (27).

Statistical Analysis

The last two observations per lipid-only, insulin-only, and the combined infusion were assumed to represent steady-state for each condition, these were averaged to yield one observation per condition per person; for the saline condition all samples were averaged for one observation per condition as steady state would be reached immediately. A second analysis using identical timing of endpoint assessments revealed the same findings (not shown). For each outcome (triglycerides, estradiol, testosterone, SHBG, NEFA, FSH, LH) a linear mixed-effects regression model was estimated parameterized with gender, condition, and the interaction between gender and condition included as fixed effects, and a random intercept, and an unstructured covariance; for estradiol and testosterone, models included only condition as a fixed effect as only males contributed to the model of testosterone, and females to the model of estradiol. The use of mixed-effects regression allows for incorporating repeated observations from all conditions per participant into one model for pairwise testing, while adjusting the variance for repeated measures, and incorporating available data from participants who did not complete the protocol. Values are expressed in text and plotted as mean (95% Confidence Interval). Triglycerides, estradiol and NEFA were analyzed on the log scale and presented as geometric mean and 95% confidence interval. P values ≤ 0.05 were considered statistically significant. No adjustments were made for multiple comparisons (28).

Results

Participation in each arm of the study

Ten males and 10 females participated in these studies and were used for the analysis. Among women, 4 completed all 4 visits, 3 completed 3 visits, 2 completed 2 visits, and 1 woman completed just one visit. Among men, 6 completed all four visits, 2 completed 3 visits, 1 completed 2 visits, and 1 completed only 1 visit. Because of the random visit order, this resulted in per-condition sample size of saline-control (9 men and 8 women), lipid alone (7 men and 9 women), insulin alone (9 men and 8 women), or lipid + insulin (8 men and 5 women).

As shown in Table 1, male and female participants were of a similar age (women 32.5 (28.2-36.8) years and men 31.4 (24.3-33.3) years, and of non-obese mean BMI. There was no evidence of insulin resistance, based on mean fasting insulin levels, fasting glucose or glucose intolerance. Hb A1c and body fat were also within normal ranges.

Hyperlipidemia and insulin resistance

In response to lipid infusion, non-esterified fatty acids (NEFA) were increased, in both males and females, to the high physiologic levels frequently observed in post-prandial metabolic syndrome patients (29). This was in sharp contrast to the NEFA levels seen with saline

infusion (Figure 2). As expected, insulin reduced NEFA levels. However, NEFA levels in the infusion of lipid plus insulin remained significantly higher than those during saline-control. The circulating TG response to the various infusions was similar for men and women. During saline-control infusion, mean TGs in men (130 (92-183) mg/dl) and women (121 (85-174) mg/dl) decreased to 57.4 (41-80) and 99 (69-143) mg/dl, respectively, with concomitant insulin infusion. During lipid infusion, TG levels increased to 227 (161-321) and 105 (74-149) mg/dl, and were 239 (167-343) and 87 (53-141) mg/dl, with concomitant insulin infusion (to convert mg/dl to mmol/L, multiply by 0.01129).

As shown in Figure 3, both sexes exhibited a significant decrease in their glucose infusion rate during the euglycemic clamp with lipid infusion, indicating development of acute insulin resistance (23, 27). Taken together, these results indicate that the elevated NEFA levels, elevated insulin levels, and insulin resistance characteristic of metabolic syndrome were achieved in both male and female subjects.

To evaluate whether the use of Liposyn or Intralipid had different effects on insulin resistance, the value of delta-glucose infusion rate, a measure of the change in insulin sensitivity, was calculated for each treatment. For Liposyn, delta-glucose infusion rate was 2.73 (1.79-3.67) mg/kg/min and for Intralipid it was 2.83 (1.06-4.60) mg/kg/min, confirming no signficant difference (p=0.9) between these two treatments in the overall cohort.

Gonadotropin response to insulin and lipid infusion

Figure 4 illustrates the effect of each infusion on serum LH levels. In women, compared to saline-control LH (4.57 (3.69-5.45) IU/L), the infusion of lipid (4.55 (3.70-5.40) IU/L) or insulin alone (3.94 (3.06-4.82) IU/L) had no effect on LH levels. However, the combination of lipid plus insulin (3.33 (2.28-4.38) IU/L) significantly decreased LH levels compared to both lipid infusion (p=0.028) and saline-control (p=0.031) (Figure 4A). Among males, LH did not differ from saline-control (3.27 (2.43-4.12) IU/L) in response to either lipid infusion (2.52 (1.60-3.44) IU/L) or insulin infusion (3.00 (2.16-3.84) IU/L). In contrast, as seen in the women, the combination of lipid plus insulin significantly decreased LH (2.18 (1.30-3.06) IU/L, p=0.02) compared to saline-control (Figure 4B)

A similar pattern was observed for FSH (Figure 5), where mean saline-control FSH levels (3.88 (3.20-4.56) IU/L) in women did not differ with infusion of lipid (4.22 (3.56-4.88) IU/L) or insulin (4.48 (3.80-5.16) IU/L) (Figure 5A). However, infusion of lipid plus insulin significantly lowered FSH (3.06 (2.29-3.83) IU/L), compared to saline-control (p=0.025), lipid alone (p=0.002) or insulin alone (p<0.001). In men (Figure 5B), serum FSH did not differ in response to infusion of lipid (1.69 (0.99-2.40) IU/L) or insulin (1.94 (1.28-2.60) IU/L), compared to saline-control (2.10 (1.44-2.76) IU/L). As in the women, the combination of lipid plus insulin significantly decreased FSH (1.47 (0.79-2.15) IU/L, p=0.03) in males compared to saline-control.

Thus, while neither agent alone significantly affected serum gonadotropins, the combination of elevated insulin and lipids, in the context of acute insulin resistance, significantly reduced LH and FSH levels in both men and women.

Sex steroids and SHBG response to insulin and lipid infusion

We further examined sex steroid and Sex Hormone Binding globulin (SHBG) responses to these short-term infusions to see if they explained any of our findings with respect to LH and FSH. As shown in Figure 6, for women, estradiol was not found to differ between the three conditions. SHBG also did not differ across all infusions, suggesting unchanged bioavailability of estradiol (Figure 6). All males studied had a testosterone level within the normal range (177-548 ng/dl) in saline-control (Manufacturer's 95% Reference Interval: 175-781ng/dl), and 151-722 ng/dl across all groups. In males, mean testosterone was significantly and equally increased, after lipid or lipid plus insulin infusion, compared to saline-control or insulin alone (Figure 6). Similar to the women, SHBG levels were not changed by any of infusions, implying that the observed differences in testosterone were not directly attributable to differential SHBG binding or bioavailability (Figure 6).

Discussion

We demonstrate herein that the combination of acutely increased circulating lipid plus insulin, under euglycemic conditions, causes a reduction in serum LH and FSH in men and women of non-obese, healthy status. The combinatorial effect of the infusion of lipid plus insulin induced a 20% or greater reduction in LH secretion in both women and men, relative to saline-control. A similar pattern was observed with the administration of lipid plus insulin on FSH levels. Thus, the acute manipulation of the metabolic milieu created by lipid plus insulin significantly impaired net pituitary gonadotropin secretion in both sexes. Alternatively, gonadotropin clearance could have been increased by the intervention. However, we have previously shown that endogenous LH clearance does not differ by body size among normally cycling women, and others have reported a prolonged half-life of endogenous LH in women of obese BMI with PCOS (26, 30). These findings provide support for the notion that the metabolic challenges of obesity translate into circulating factors that adversely affect pituitary function, and support our previous findings that obesity related reproductive function is, at least in part, linked to pituitary dysfunction (6).

Effects of hyperinsulinemia on the pituitary gland are unclear, in part because it is not known whether or not the pituitary gland is capable of acquiring the characteristic insulin resistance observed in liver and muscle of individuals with obesity. Studies using the pituitary insulin receptor knockout (pitIRKO) mouse indicate that the pituitary likely remains insulin sensitive in the face of obesity and its metabolic challenges (31). It is tempting to speculate that excess insulin action in the pituitary, when exposed to increased free fatty acids, causes their rapid incorporation into the cell, thereby inducing a lipotoxic endoplasmic reticulum stress and induction of the unfolded protein response (32), and pausing gonadotropin gene translation.

Although our sample size is too small to draw firm conclusions, these findings suggest a possible sexual dimorphism in HPG axis function between women and men when exposed to a high fat environment. Lipid infusion was associated with a short-term, but small, increase in testosterone in men, and a trend for a small decrease in estradiol in women. It is possible that the reduction in gonadotropins in males undergoing lipid plus insulin infusion may have been a result of negative feedback of this small increase in testosterone. However,

all testosterone levels remained within the normal range. Nonetheless, the short-term effect of this infusion did not recapitulate hypogonadism in men or women, as neither testosterone nor estradiol were acutely lowered. An effect of lipid or insulin on testosterone would not be expected to be observed in women, because of the overriding effect of estradiol on the female HP axis and the much lower circulating testosterone levels. However, reduction of androgen activity in women with flutamide has been shown to improve the lipid profile in women with PCOS (33). Regardless, the high, supraphysiologic concentrations of insulin and lipid provided in this study are far more likely than the modest elevation of testosterone to have accounted for the negative feedback we observed in men. Unsurprisingly, SHBG, which has a long serum half-life, was unaltered in concentration over the course of these short-term infusions.

Strengths of our study include the carefully controlled conditions of each infusion with excellent achievement and stability of elevated insulin and NEFA levels during each infusion. The repeated nature of the study design, with the same subjects participating in up to 4 treatment arms is also a strength. Weaknesses of our study include its relatively small sample size, multiple comparisons, a lack of very frequent blood sampling (q 10 minutes) required to allow assessment of pulsatile gonadotropin secretion, and the fact that not all participants were able to complete all infusion types.

In conclusion, the combination of acute exposure to elevated fatty acids and hyperinsulinemia acutely down regulates pituitary gonadotropin levels in both women and men. Thus, mimicking metabolic syndrome in individuals characterized as healthy and non-obese, can induce the reproductive phenotype characteristic of obesity.

Acknowledgments

The authors would like to thank Ms. Leah Herlache Vanberg for study coordination and technical assistance and the study participants.

Funding: This work was supported by NIH grants U54 HD058155 (Jeffrey Pollard, PhD, PI), K23 DK091553 (Irene Schauer, PI), K12 HD057022 (Judy Regensteiner and Nanette Santoro, co-PIs), VA Merit Award (Jane Reusch, PI), and TL1 TR001081 (Ron Sokol, MD, PI).

References

1. Fedorcsak P, Storeng R, Dale PO, Tanbo T, Abyholm T. Obesity is a risk factor for early pregnancy loss after IVF or ICSI. Acta obstetricia et gynecologica Scandinavica. 2000; 79:43–48. [PubMed: 10646815]
2. Gesink Law DC, Maclehose RF, Longnecker MP. Obesity and time to pregnancy. Hum Reprod. 2007; 22:414–420. [PubMed: 17095518]
3. Linne Y. Effects of obesity on women's reproduction and complications during pregnancy. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2004; 5:137–143. [PubMed: 15245382]
4. Polotsky AJ, Hailpern SM, Skurnick JH, Lo JC, Sternfeld B, Santoro N. Association of adolescent obesity and lifetime nulliparity--the Study of Women's Health Across the Nation (SWAN). Fertility and sterility. 2010; 93:2004–2011. [PubMed: 19185860]
5. Jain A, Polotsky AJ, Rochester D, Berga SL, Loucks T, Zeitlian G, et al. Pulsatile luteinizing hormone amplitude and progesterone metabolite excretion are reduced in obese women. The Journal of clinical endocrinology and metabolism. 2007; 92:2468–2473. [PubMed: 17440019]

6. Al-Safi ZA, Liu H, Carlson NE, Chosich J, Lesh J, Robledo C, et al. Estradiol Priming Improves Gonadotrope Sensitivity and Pro-inflammatory Cytokines in Obese Women. The Journal of clinical endocrinology and metabolism. 2015:jc20151946.
7. Al-Safi ZA, Polotsky AJ. Obesity and menopause. Best Pract Res Clin Obstet Gynaecol. 2015; 29:548–553. [PubMed: 25579233]
8. Grenman S, Ronnemaa T, Irjala K, Kaihola HL, Gronroos M. Sex steroid, gonadotropin, cortisol, and prolactin levels in healthy, massively obese women: correlation with abdominal fat cell size and effect of weight reduction. The Journal of clinical endocrinology and metabolism. 1986; 63:1257–1261. [PubMed: 3097052]
9. Pagan YL, Srouji SS, Jimenez Y, Emerson A, Gill S, Hall JE. Inverse relationship between luteinizing hormone and body mass index in polycystic ovarian syndrome: investigation of hypothalamic and pituitary contributions. The Journal of clinical endocrinology and metabolism. 2006; 91:1309–1316. [PubMed: 16434454]
10. Santoro N, Lasley B, McConnell D, Allsworth J, Crawford S, Gold EB, et al. Body size and ethnicity are associated with menstrual cycle alterations in women in the early menopausal transition: The Study of Women's Health across the Nation (SWAN) Daily Hormone Study. The Journal of clinical endocrinology and metabolism. 2004; 89:2622–2631. [PubMed: 15181033]
11. Sherman BM, Korenman SG. Measurement of serum LH, FSH, estradiol and progesterone in disorders of the human menstrual cycle: the inadequate luteal phase. The Journal of clinical endocrinology and metabolism. 1974; 39:145–149. [PubMed: 4835128]
12. Downing JA, Scaramuzzi RJ. The effect of the infusion of insulin during the luteal phase of the estrous cycle on the ovulation rate and on plasma concentrations of LH, FSH and glucose in ewes. Theriogenology. 1997; 47:747–759. [PubMed: 16728025]
13. Williams NI, Lancas MJ, Cameron JL. Stimulation of luteinizing hormone secretion by food intake: evidence against a role for insulin. Endocrinology. 1996; 137:2565–2571. [PubMed: 8641210]
14. Lawson MA, Jain S, Sun S, Patel K, Malcolm PJ, Chang RJ. Evidence for insulin suppression of baseline luteinizing hormone in women with polycystic ovarian syndrome and normal women. The Journal of clinical endocrinology and metabolism. 2008; 93:2089–2096. [PubMed: 18334581]
15. Adashi EY, Hsueh AJ, Yen SS. Insulin enhancement of luteinizing hormone and follicle-stimulating hormone release by cultured pituitary cells. Endocrinology. 1981; 108:1441–1449. [PubMed: 6781875]
16. Sharma S, Morinaga H, Hwang V, Fan W, Fernandez MO, Varki N, et al. Free fatty acids induce Lhb mRNA but suppress Fshb mRNA in pituitary LbetaT2 gonadotropes and diet-induced obesity reduces FSH levels in male mice and disrupts the proestrous LH/FSH surge in female mice. Endocrinology. 2013; 154:2188–2199. [PubMed: 23525221]
17. Schauer IE, Herlache L, Regensteiner JG, Reusch JEB. Induction of insulin resistance by acute fatty acid elevation partially recapitulates exercise defects seen in diabetes. Journal of Womens Health. 2009; 18:1502.
18. Schauer IE, Herlache LL, Regensteiner JG, Reusch JEB. Effect of acute induction of insulin resistance on exercise parameters. Diabetes. 2012; 61(Suppl 1):A49.
19. Widom B, Diamond MP, Simonson DC. Alterations in glucose metabolism during menstrual cycle in women with IDDM. Diabetes Care. 1992; 15:213–220. [PubMed: 1547678]
20. Ritter O, Jelenik T, Roden M. Lipid-mediated muscle insulin resistance: different fat, different pathways? J Mol Med (Berl). 2015; 93:831–843. [PubMed: 26108617]
21. Nowotny B, Zahiragic L, Krog D, Nowotny PJ, Herder C, Carstensen M, et al. Mechanisms underlying the onset of oral lipid-induced skeletal muscle insulin resistance in humans. Diabetes. 2013; 62:2240–2248. [PubMed: 23454694]
22. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979; 237:E214–223. [PubMed: 382871]
23. Bergman BC, Howard D, Schauer IE, Maahs DM, Snell-Bergeon JK, Clement TW, et al. The importance of palmitoleic acid to adipocyte insulin resistance and whole-body insulin sensitivity in type 1 diabetes. The Journal of clinical endocrinology and metabolism. 2013; 98:E40–50. [PubMed: 23150678]

24. Roth LW, Allshouse AA, Bradshaw-Pierce EL, Lesh J, Chosich J, Kohrt W, et al. Luteal phase dynamics of follicle-stimulating and luteinizing hormones in obese and normal weight women. Clinical endocrinology. 2014
25. Levine JA. Measurement of energy expenditure. Public Health Nutr. 2005; 8:1123–1132. [PubMed: 16277824]
26. Roth LW, Bradshaw-Pierce EL, Allshouse AA, Lesh J, Chosich J, Bradford AP, et al. Evidence of GnRH antagonist escape in obese women. The Journal of clinical endocrinology and metabolism. 2014; 99:E871–875. [PubMed: 24650013]
27. Schauer IE, Snell-Bergeon JK, Bergman BC, Maahs DM, Kretowski A, Eckel RH, et al. Insulin resistance, defective insulin-mediated fatty acid suppression, and coronary artery calcification in subjects with and without type 1 diabetes: The CACTI study. Diabetes. 2011; 60:306–314. [PubMed: 20978091]
28. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990; 1:43–46. [PubMed: 2081237]
29. Shojaee-Moradie F, Ma Y, Lou S, Hovorka R, Umpleby AM. Prandial hypertriglyceridemia in metabolic syndrome is due to an overproduction of both chylomicron and VLDL triacylglycerol. Diabetes. 2013; 62:4063–4069. [PubMed: 23990358]
30. Srouji SS, Pagan YL, D'Amato F, Dabela A, Jimenez Y, Supko JG, et al. Pharmacokinetic factors contribute to the inverse relationship between luteinizing hormone and body mass index in polycystic ovarian syndrome. The Journal of clinical endocrinology and metabolism. 2007; 92:1347–1352. [PubMed: 17264175]
31. Brothers KJ, Wu S, DiVall SA, Messmer MR, Kahn CR, Miller RS, et al. Rescue of obesity-induced infertility in female mice due to a pituitary-specific knockout of the insulin receptor. Cell Metab. 2010; 12:295–305. [PubMed: 20816095]
32. Kim T, Do MH, Lawson MA. Translational control of gene expression in the gonadotrope. Mol Cell Endocrinol. 2014; 385:78–87. [PubMed: 24035865]
33. Diamanti-Kandarakis E, Mitrakou A, Raptis S, Tolis G, Duleba AJ. The effect of a pure antiandrogen receptor blocker, flutamide, on the lipid profile in the polycystic ovary syndrome. The Journal of clinical endocrinology and metabolism. 1998; 83:2699–2705. [PubMed: 9709934]

Study Importance Questions

- Obesity is associated with hyperlipidemia, insulin resistance, and relative hypogonadotropic hypogonadism.
- The etiologies of obesity-linked reproductive dysfunction are not well understood.
- In healthy individuals characterized as non-obese, euglycemic hyperinsulinemia combined with elevated lipids acutely suppresses LH and FSH.
- Acute mimicking of metabolic syndrome in individuals characterized as non-obese and healthy reproduced the reproductive phenotype of obesity via gonadotropin suppression, despite an increase in testosterone in men.
- Study provides a possible mechanism underlying the relative hypogonadotropic hypogonadism of obesity.

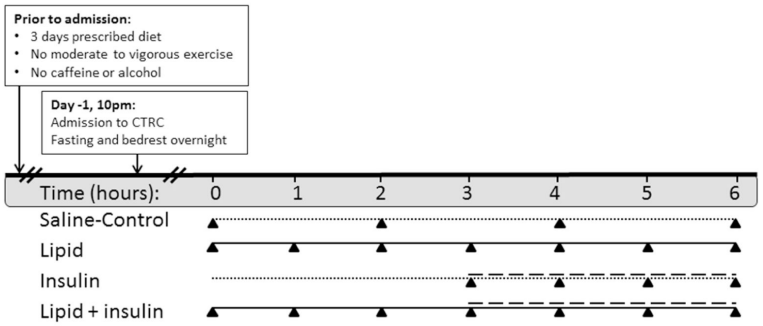

Figure 1.

Depiction of each study arm protocol and timeline. Infusions were conducted at independent visits. T=0 was approximately 6AM for all visits. Dotted line denotes saline-control infusions, solid line lipid/heparin infusion and dashed line indicates insulin infusion for hyperinsulinemic euglycemic clamp. Triangles denote blood draws; T=0 blood sample was obtained immediately prior to start of infusions.

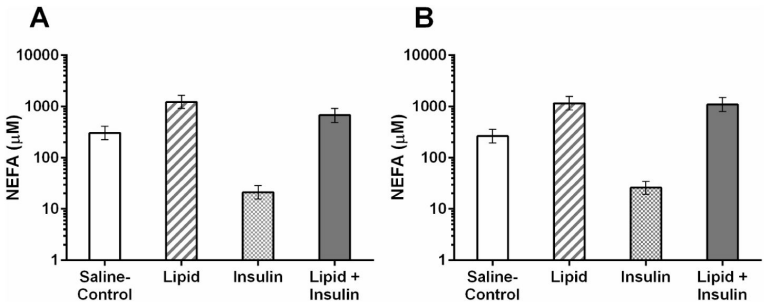

Figure 2.

Serum non-esterified fatty acids (NEFA) levels in response to the saline, lipid, insulin or lipid + insulin infusions in (A) females and (B) males. Note that insulin infusion leads to a physiologic decrease in circulating NEFAs despite their continued infusion. Data are geometric means and 95% confidence intervals plotted on the log scale. All pairwise comparisons are statistically significant ($p<0.003$) with the exception of lipid *vs* lipid + insulin in males ($p=0.79$)

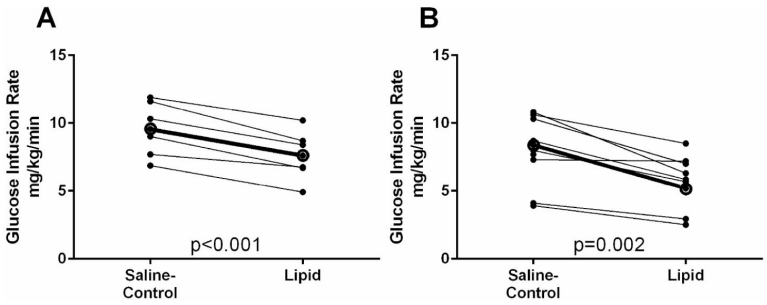

Figure 3.

Glucose infusion rate (GIR) in response to saline-control and lipid infusions in (A) females and (B) males during the last 30min of the hyperinsulinemic euglycemic clamp. Note the significantly decreased glucose uptake (decrease in infusion rate needed to maintain euglycemia) in the presence of high circulating NEFAs in both sexes. Bold lines indicate mean GIR for male and female subjects.

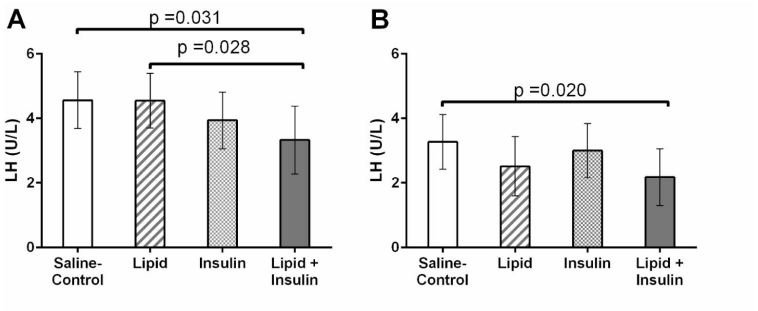

Figure 4.

Serum LH levels in (A) females and (B) males in response to the saline, lipid, insulin or lipid + insulin infusions. Data are means and 95% confidence intervals. Where the pairwise $p < 0.05$ a p-value is provided.

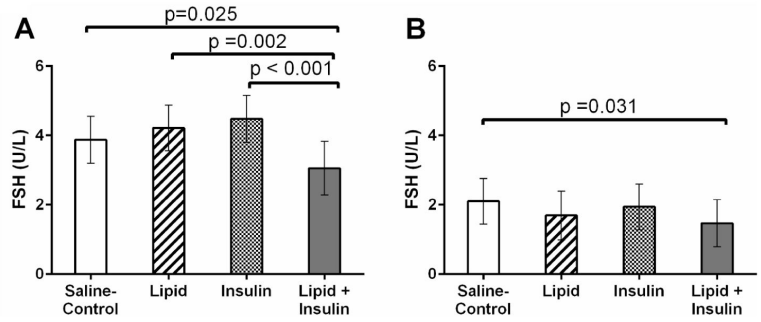

Figure 5.

Serum FSH levels in (A) females and (B) males in response to the saline, lipid, insulin or lipid + insulin infusions. Data are means and 95% confidence intervals. Where the pairwise $p < 0.05$ a p-value is provided.

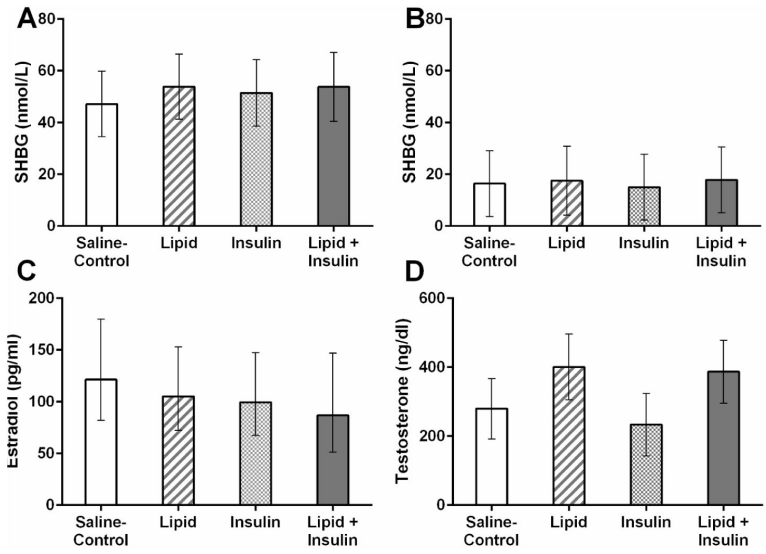

Figure 6.

Effects of infusions on sex steroids and sex steroid binding protein (SHBG) levels in females (A and C) and males (B and D). Data are means and 95% confidence intervals (geometric mean for Estradiol). In panel D, significant differences were: saline-control vs lipid or lipid + insulin p=0.02, insulin vs lipid p=0.002, insulin vs lipid + insulin p=0.004. To convert estradiol to pmol/L, multiply by 3.671; to convert testosterone to nmol/L, multiply by 0.0347.

Table 1

Characteristics of the study participants.

	Females	**Males**
N	10	10
Age (Yrs)	32.5 ± 6.0	28.8 ± 6.3
BMI (kg/m^2)	22.8 ± 2.6	24.7± 2.7
Body fat % (DEXA)	30.2 ± 4.7	22.3 ± 6.7
Fasting glucose (mg/dl)	81.2 ± 6.8	85.5 ± 3.1
2hr glucose (mg/dl)	76.9_± 17.9	76.3 ± 23.1
Hemoglobin A1c (%)	5.4 ± 0.4	5.2 ± 0.3
Fasting insulin (μU/ml)	7.0 ± 3.1	6.2 ± 4.2

To convert fasting glucose to mmol/L, multiply by 0.0555; to convert insulin to pmol/L, multiply by 6.945. Data are mean ± standard deviation.