Decreasing Insulin Sensitivity in Women Induces Alterations in LH Pulsatility

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Context: Obesity is associated with neuroendocrine reproductive alterations and decreased fertility.

Objective: The objective of the study was to gain insight into the neuroendocrine mechanisms implicated in these alterations.

Design: The effects on pulsatile LH secretion of 28 days of a hypercaloric diet were studied in lean and regularly cycling female volunteers. Approximately 50% extra calories (3 g sucrose/kg body weight per day and 1 g fat/kg body weight per day) were added to their individual daily requirements. Spontaneous and insulin-stimulated LH secretion was recorded on 2 different days, before and at the end of the caloric load.

Results: The hypercaloric diet induced an average weight gain of 2.0 $\pm$ 0.3 kg ($P < .05$), corresponding to a body mass index increase of 0.7 $\pm$ 0.1 kg/m^2 ($P < .05$). A concomitant decrease of 11.6% $\pm$ 4.6% in whole-body insulin sensitivity was also observed ($\delta = -1.6 \pm 0.7$ mg/kg $\cdot$ min glucose; $P < .05$). The frequency of spontaneous and insulin-stimulated pulsatile LH secretion was increased by 17.9% $\pm$ 9.0% and 26.5% $\pm$ 9.0%, respectively (both $P < .05$). Spontaneous LH peak amplitude was decreased by 26.5% $\pm$ 9.0% ($\delta = -0.7 \pm 0.36$ U/L; $P < .05$), a change correlated with insulin sensitivity.

Conclusions: Short-term weight gain in normal female volunteers induces alterations of LH secretion reminiscent to those observed in obesity. A decrease in insulin sensitivity may constitute a mechanistic link between obesity and its associated neuroendocrine dysfunctions. **(*J Clin Endocrinol Metab* 101: 3240–3249, 2016)**

The activity of the female neuroendocrine reproductive axis is closely associated with nutritional status. This relationship was first established in rodents (1) and then subsequently demonstrated in other animal models and in humans (2–6). In the human, Frisch and McArthur (5) postulated that a minimum amount of body fat is necessary to allow the onset and the maintenance of regular menstrual cycles. Consistently, states of low or insufficient energy availability, such as encountered in anorexia nervosa or overexercising, can also lead to hypothalamic amenorrhea (7–9), and it is generally well accepted that insufficient nutrition negatively impacts the activity of the neuroendocrine reproductive axis.

Weight excess and obesity also appear deleterious for reproduction (10–13). However, compared with weight insufficiency, the mechanisms implicated here are far less understood. The first evidence indicating that excessive nutritional intake could be associated with reduced fertility was a population-based analysis conducted in more than 60 countries (14). This work suggested that extreme

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Abbreviations: BMI, body mass index; CNS, central nervous system; HOMA-IR, insulin resistance according to the homeostatic model assessment; PCOS, polycystic ovary syndrome.

conditions of either very low or very high caloric intake are both associated with poor reproductive outcome. The hypothesis that excessive weight can impact negatively upon the reproductive function is supported by several subsequent studies. Zaadstra et al (15) showed in a cohort of 500 healthy women seeking insemination for male infertility that their waist to hip ratio was inversely correlated to their conception rate. Similarly, in two retrospective studies including large numbers of individuals, the relative risk of infertility was found to increase with the body mass index (BMI) of the female or of both partners (12, 16). Consistently, in a prospective study of 3029 couples with unexplained infertility followed up for 2 years, women with a BMI greater than 28 kg/m^2 had a 4% decrease in spontaneous pregnancy rate per unit increase in BMI (13). However, these epidemiological data do not provide any mechanistic explanation.

In rodents, high-fat-fed obese female mice displayed lower fertility than the controls fed a normal chow, a phenotype associated with a down-regulation of hypothalamic GnRH expression (17). In humans, polycystic ovary syndrome (PCOS) is an endocrine condition associating overweight or obesity with a reproductive phenotype characterized by dysfunctions at the neuroendocrine and the ovarian levels (18–20). Peripheral insulin resistance, a hallmark of PCOS, has been implicated in the pathogenesis of the syndrome. Insulin, like leptin (21, 22), is a metabolic signal involved in the central nervous system (CNS) regulation of body weight and reproduction (23–25). This dual function is illustrated by the phenotype of neuron-specific insulin receptor knockout mice, which harbor a neuron-specific deletion of the insulin receptor gene (23). These mice have complete insulin resistance in the CNS and develop hyperphagic obesity associated with hypogonadism of hypothalamic origin. The latter observation suggests that in mice, activation of GnRH neurons is dependent on adequate insulin signaling within the hypothalamus. This hypothesis is consistent with our previous in vivo (26) and in vitro data, demonstrating that in rodents, hypothalamic GnRH neurons are indeed insulin sensitive (27, 28). Because we could also demonstrate that in normal young women, pulsatile secretion of LH is modulated by insulin (29), we hypothesized that insulin could play a pathophysiological role in the reproductive phenotype of PCOS.

Therefore, the aim of this study was to further explore the interaction between insulin and the control of reproduction in humans. We used a model of a short-term hypercaloric diet to test the hypothesis that changes in whole-body insulin sensitivity can modify the secretion of neuroendocrine reproductive hormones in humans. Given the close association between obesity and insulin sensitivity, a better understanding of the relationship of the latter with fertility could become very important in the work-up and treatment of obesity and its related metabolic disorders.

Materials and Methods

Study subjects

The study was approved by the local ethics committee and all volunteers provided written informed consent. Subjects were recruited by poster campaign. Between June 2013 and February 2014, 107 potential subjects were evaluated by telephone screenings, 27 of whom were identified as eligible for the screening visit, and 13 finally included in the study. All anthropometrics measurements were recorded at 7:30 AM on the test days, after an overnight fast. Height was measured at the screening visit, and body weight was assessed at each visit.

Four subjects discontinued the study for the following reasons: irregular menstrual cycles (n = 1), failure to adhere to the protocol (n = 2), and occurrence of hypokalemia during insulin infusion (n = 1). Thus, nine subjects completed the study and were included in our analyses. After completing the study, all the volunteers were offered dietary consultations to help them restore their baseline weight.

Study design

A hypercaloric feeding protocol lasting 28 days was used to explore the effects of weight gain and decreased whole-body insulin sensitivity on neuroendocrine reproductive hormones. To this end, pulsatile LH secretion was used as a surrogate marker of the activity of hypothalamic GnRH neurons (30, 31). The spontaneous and the insulin-stimulated LH secretion profiles were evaluated twice: the first time under strict conditions of a controlled isocaloric diet and the second time at the end of 28 days of hypercaloric feeding. The evaluations of LH secretion were always performed in the follicular phase of the menstrual cycle, according to our previously published protocols (29).

Nutritional interventions

The controlled isocaloric diet was initiated 3 days before the anticipated first day of the next menstrual cycle and was designed to last until completion of the 2 test days described below (~7 d, depending on the occurrence of menstruations). The energetic content of the isocaloric diet was calculated to provide 1.5 times the resting metabolic rate measured by indirect calorimetry (32, 33). The nutritional composition of the isocaloric diet was 55% carbohydrates, 15% proteins, and 30% fat.

The hypercaloric diet was designed to last 28 days in total and was initiated 24 days before the expected day of the next menstrual cycle. During the initial 21 days of voluntary high-calorie intake, subjects were instructed to add a supplement of sucrose (3 g/kg · d, diluted in 1 L of water) and lipids (1 g/kg · d, two-thirds butter and one-third olive oil) to their ad libitum diet. Three days before the expected day of the next menstrual cycle, they were switched for an additional 7 days to controlled hypercaloric feeding, consisting of an isocaloric diet supplemented with 3 g/kg · d of sucrose and 1 g/kg · d of lipids. During that period, we provided all necessary food to the volunteers, who were asked to restrain from eating anything else. Controlled feeding was ad-

opted during the last week of the intervention to normalize the nutritional intake at the time of the tests. Also, volunteers were asked to avoid all strenuous physical activity during the entire overfeeding period. The two nutritional interventions (isocaloric and hypercaloric feeding) were separated by at least 1 month.

Test days

At the end of each intervention (isocaloric or hypercaloric feeding), volunteers were admitted twice to the Clinical Research Center of the University Hospital of Lausanne, the first time for a clamp day and the second time for a fasting test day. All test days consisted of frequent (every 10 min) blood sampling and were started at 7:00 AM with the admission of the volunteer after an overnight fast. On the fasting days, a single catheter was inserted into a forearm vein, and frequent blood sampling was started after 30 minutes of rest. The total duration of the protocol was 10 hours, during which volunteers were kept fasted. Body composition was evaluated by a bioimpedance analysis (Imp DF50; ImpediMed) during the day.

For the clamp days, a second catheter was inserted into a contralateral forearm vein to infuse insulin and glucose. During clamp days, subjects underwent the same frequent blood sampling protocol as the fasting day, this time together with a hyperinsulinemic euglycemic clamp lasting throughout the 10 hours of sampling. Clamp days were performed first, between day 2 and 7 of the menstrual cycle, and fasting days took place exactly 3 days later. The order of the test days was not randomized to avoid programming a full day of fasting before the clamp. All blood samples were immediately centrifuged upon collection, and serum was frozen on-site.

Hyperinsulinemic and euglycemic clamps were performed according to our previously published protocol (29). A bolus of insulin was injected 30 minutes after the insertion of the catheters, followed by a constant infusion at a rate of 1 mU/kg · min, which was continued for 10 hours. Blood samples were obtained every 5 minutes for the assessment of glycemia, using two different measures obtained on-site on a glucometer (Accu-chek Aviva; Roche). A variable infusion of 20% glucose allowed clamping of glycemia at 5.5 mmol/L (34).

Assays and data analysis

LH, FSH, and insulin were measured by an immunoenzymmologic assay (COBAS; Roche Diagnostics International AG). Leptin and grehlin were measured by an ELISA and adiponectin by a multiplex analysis, using commercially available kits and reagents (Merck Millipore AG). Estradiol, progesterone, and T were measured by chemiluminescent microparticle immunoassay (Architect i2000SR; Abbott AG). Glucose was measured by hexokinase; cholesterol by cholesterol-oxidase and p-aminoantipyrine; high-density lipoprotein cholesterol by cholesterol-oxidase, p-aminoantipyrine, and homogeny polyethylene glycol; low-density lipoprotein cholesterol calculated with Friedewald formula; high-sensitivity C-reactive protein by immunoturbidimetry; and triglycerides by glycerol phosphate oxidase p-aminoantipyrine (all on COBAS; Roche Diagnostics International AG).

The characteristics of LH pulsatility were analyzed by a modified Santen and Bardin method (35). LH pulse amplitude was calculated as the difference between the nadir and the highest peak within 30 minutes of the nadir. Pulse amplitudes during a given admission were averaged for each subject. Whole-body insulin sensitivity was expressed as the mean rate of glucose infusion necessary to maintain euglycemia for the last 8 hours of the clamps. Insulin resistance according to the homeostatic model assessment (HOMA-IR) was calculated using the standard formula (36). Statistical analyses were completed using Wilcoxon's nonparametric signed-rank test for the between-group comparison and the nonparametric Spearman's correlation method for the correlation analysis. Power analyses were completed using SAS Power and Sample Size software (SAS Inc).

Results

Study subjects and design

The subjects were women aged between 18 and 30 years (mean age 23.6 ± 0.8 y), with regular menstrual cycles of 30.2 ± 0.8 days and a mean BMI of 21.9 ± 0.7 kg/m^2 (mean weight of 60.5 ± 2.2 kg). Their mean body fat content was 24.1% ± 2.5%. Mean fasting glycemia was 4.6 ± 0.1 mmol/L, with a mean fasting insulin of 5.2 ± 0.7 mU/L and a mean glycated hemoglobin level of 5.3% ± 0.1%. These parameters were assessed at the screening visit between day 4 and day 9 of the menstrual cycle and confirmed that all the variables were within the normal range at baseline. In addition, they were not pregnant, did not take hormonal contraception, were not participating in sports for more than 4 hours a week, did not smoke more than 10 cigarettes per day, and did not drink more than three alcoholic beverages per day. A complete medical history did not identify any hormonal problem, and physical examinations were unremarkable.

The individual duration of the dietary intervention was somewhat variable, depending on the length of the menstrual cycle. Thus, the controlled isocaloric diet lasted 7.1 ± 0.7 days, the average time interval between the isocaloric and the hypercaloric phases was 62.5 ± 11 days, and the length of the hypercaloric phase (voluntary overfeeding + controlled hypercaloric diet) was 29.4 ± 0.8 days, of which 7.6 ± 0.6 were a controlled diet.

Metabolic data

The hypercaloric diet induced a mean weight gain of 2.0 ± 0.3 kg, with most participants returning to their baseline weight by the final visit (Figure 1A). The subject with the lowest baseline BMI gained the least amount of weight (BMI 18.6 kg/m^2; δ weight 0.7 kg), whereas the subject with the highest baseline BMI gained the most weight (BMI 24.9 kg/m^2; δ weight 4.0 kg, Figure 1B). Of note, the latter subject was also the only participant who had not returned to her inclusion weight by the final visit. The evolution of the volunteers' BMI followed the same pattern, with the hypercaloric diet inducing a mean increase of 0.7 ± 0.1 kg/m^2 ($P < .05$). We also found a

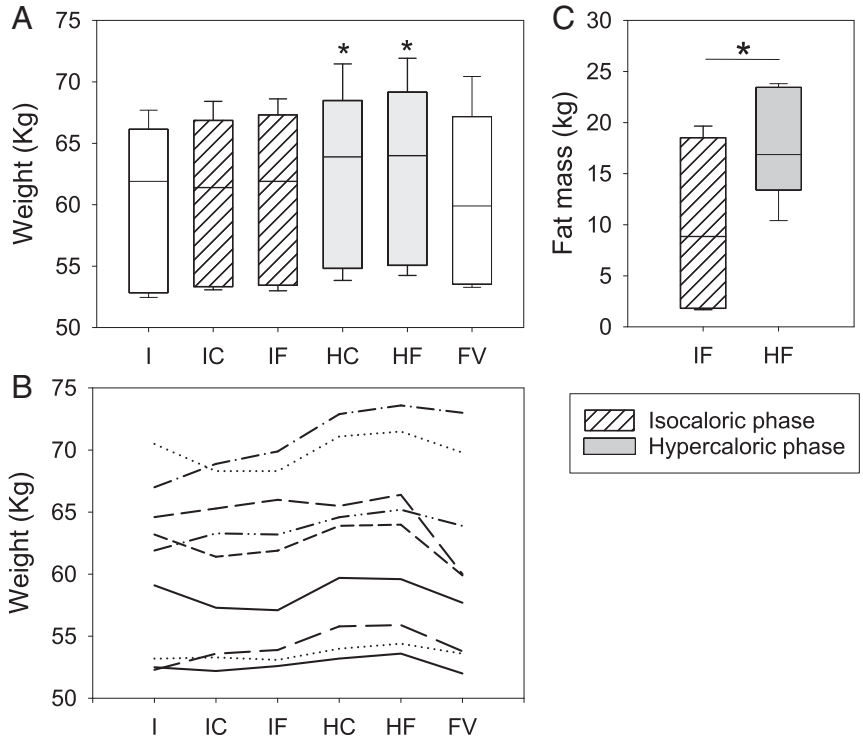

Figure 1. Weight and body adiposity. A, Weight of the volunteers at the different time points indicated. B, Evolution of individual weights of the nine volunteers. C, Fat mass before and at the end of hypercaloric diet. FV, final visit; HC, hypercaloric clamp day; HF, hypercaloric fasting day; I, inclusion; IC, isocaloric clamp day; IF, isocaloric fasting day. *, $P < .05$. Data are represented as median, 10th, 25th, 75th, and 90th percentiles in panels A and C.

significant increase of 3.8 ± 1.5 kg in absolute fat mass at the end of the hypercaloric diet ($P < .05$, Figure 1C), translating into a relative increase of 5.2% ± 2.1% ($P < .05$).

These changes in body weight and composition were accompanied by a significant decrease in whole-body insulin sensitivity. The mean glucose infusion rate necessary to maintain euglycemia during the insulin clamp performed in isocaloric conditions was 12.1 ± 3.0 mg/kg · min, compared with 10.4 ± 2.4 mg/kg · min at the end of the hypercaloric diet. The latter value, albeit still within the normal range, was significantly lower than the former ($\delta = -1.57 \pm 2$ mg/kg · min; $P < .05$). Insulin resistance according to the HOMA-IR also increased significantly between the isocaloric and the hypercaloric phase (1.1 ± 0.2 vs 1.5 ± 0.2, respectively; $\delta = 0.4 \pm 0.1$; $P < .05$). Evaluations of circulating metabolites showed that total cholesterol, high-density lipoprotein cholesterol, fasting glucose, and insulin levels all increased significantly with the hypercaloric diet (Supplemental Table 1). Leptin and adiponectin were also significantly increased by hypercaloric feeding, whereas ghrelin was not affected (data not shown).

Menstrual cycle and reproductive hormones

The clamp test days took place during the early follicular phase (d 4.3 ± 0.5 of the cycle in isocaloric condition, and 5.0 ± 0.4 in hypercaloric condition). The fasting test days, programmed 3 days after the clamps, occurred significantly later in the cycle (d 7.3 ± 0.5 in isocaloric condition, and 8.0 ± 0.4 in hypercaloric condition). The occurrence of a progression in the cycle between the 2 test days is also supported by the rising levels of LH and estradiol observed in the isocaloric phase (Table 1).

LH secretion profiles

Figure 2 displays the spontaneous LH secretion profile of each volunteer at the end of isocaloric and hypercaloric feeding. In eight of the nine volunteers, the LH pulse frequency on the isocaloric fasting test day was between six and nine peaks in 10 hours. A single volunteer (number 8) had only one LH peak. At the end of the hypercaloric feeding, the LH pulse frequency was increased in six individuals, unchanged in two, and decreased (from eight to seven in 10 h) in a single individual (number 2). Similarly, the mean LH pulse amplitude was between 0.83 and 2.43 U/L for all

Table 1. Timing of the Various Test Days With Respect to the Menstrual Cycle (d 1 Defined as the First Day of Menstruations), With the Baseline Levels of Reproductive Hormones

	IC	IF	HC	HF	P^1	P^2
Day of cycle	4.3 ± 0.5	7.3 ± 0.5	5 ± 0.4	8 ± 0.4	<.01	<.01
Estradiol, nmol/L	0.13 ± 0.01	0.17 ± 0.03	0.16 ± 0.01	0.22 ± 0.03	<.05	ns
LH, U/liter)	3.53 ± 0.54	5.81 ± 0.66	5.31 ± 0.43	6.36 ± 0.57	<.05	ns
FSH, U/L	4.91 ± 0.4	5.26 ± 0.38	5.4 ± 0.52	5.46 ± 0.42	ns	ns
T, nmol/L	0.87 ± 0.06	0.97 ± 0.07	0.88 ± 0.08	1.01 ± 0.08	ns	ns

Abbreviations: IC, isocaloric clamp day; IF, isocaloric fasting day; HC, hypercaloric clamp day; HF, hypercaloric fasting day; ns, not significant.
P^1 indicates a difference between IC and IF, and P^2 indicates a difference between HC and HF. Data are expressed as mean ± SEM.

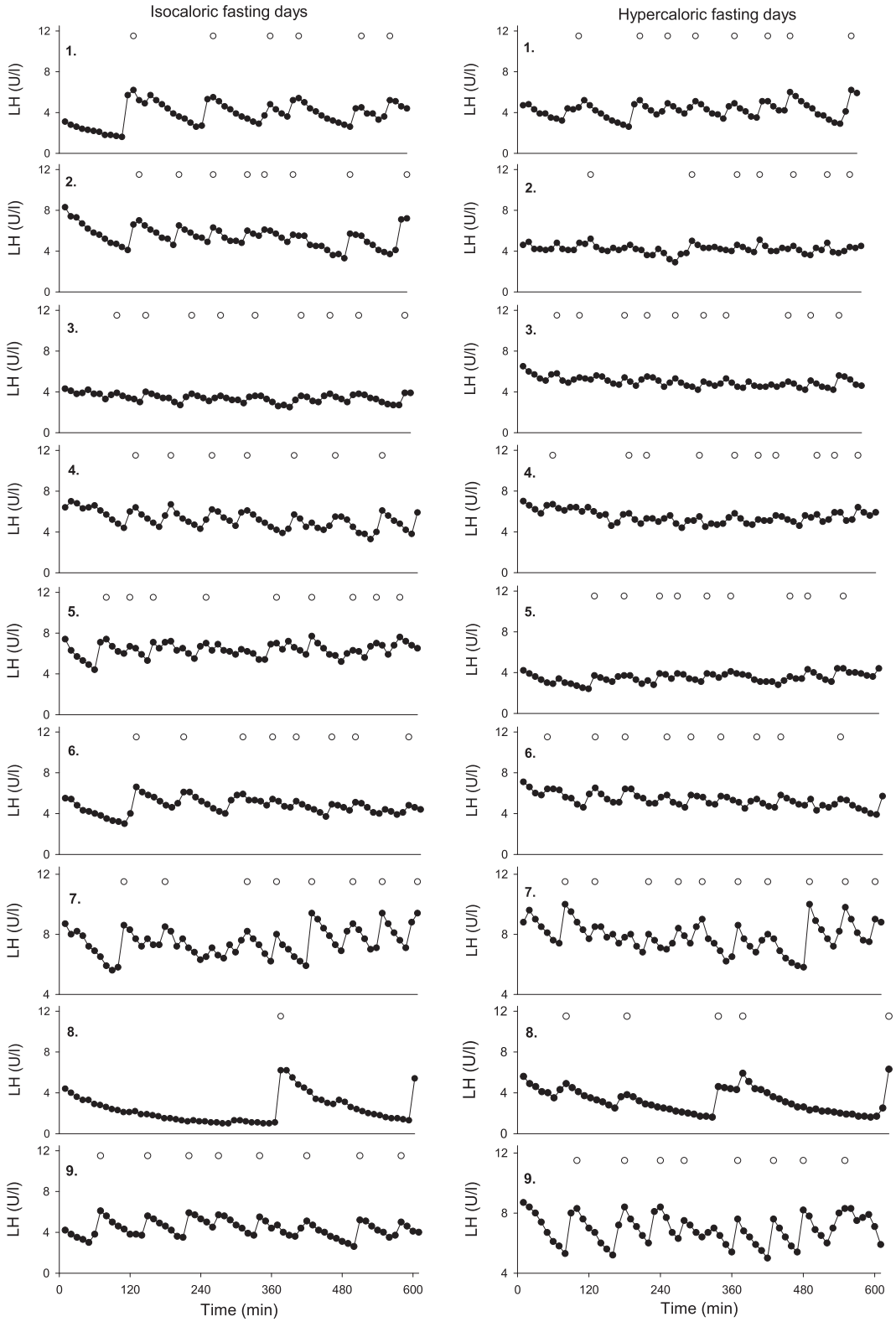

Figure 2. Individual profiles of spontaneous LH secretion during IF (left) and IC (right). Open circles indicate significant peaks. IC, isocaloric clamp day; IF, isocaloric fasting day.

volunteers except subject number 8, who had a single peak with an amplitude of 5.2 U/L. At the end of the hypercaloric feeding, the mean LH pulse amplitude was decreased in all subjects but one (number 9), in whom the amplitude increased from 2.0 to 2.46 U/L. Of note, subject number 9 was also the only subject showing an increase in whole-body insulin sensitivity at the end of the hypercaloric diet.

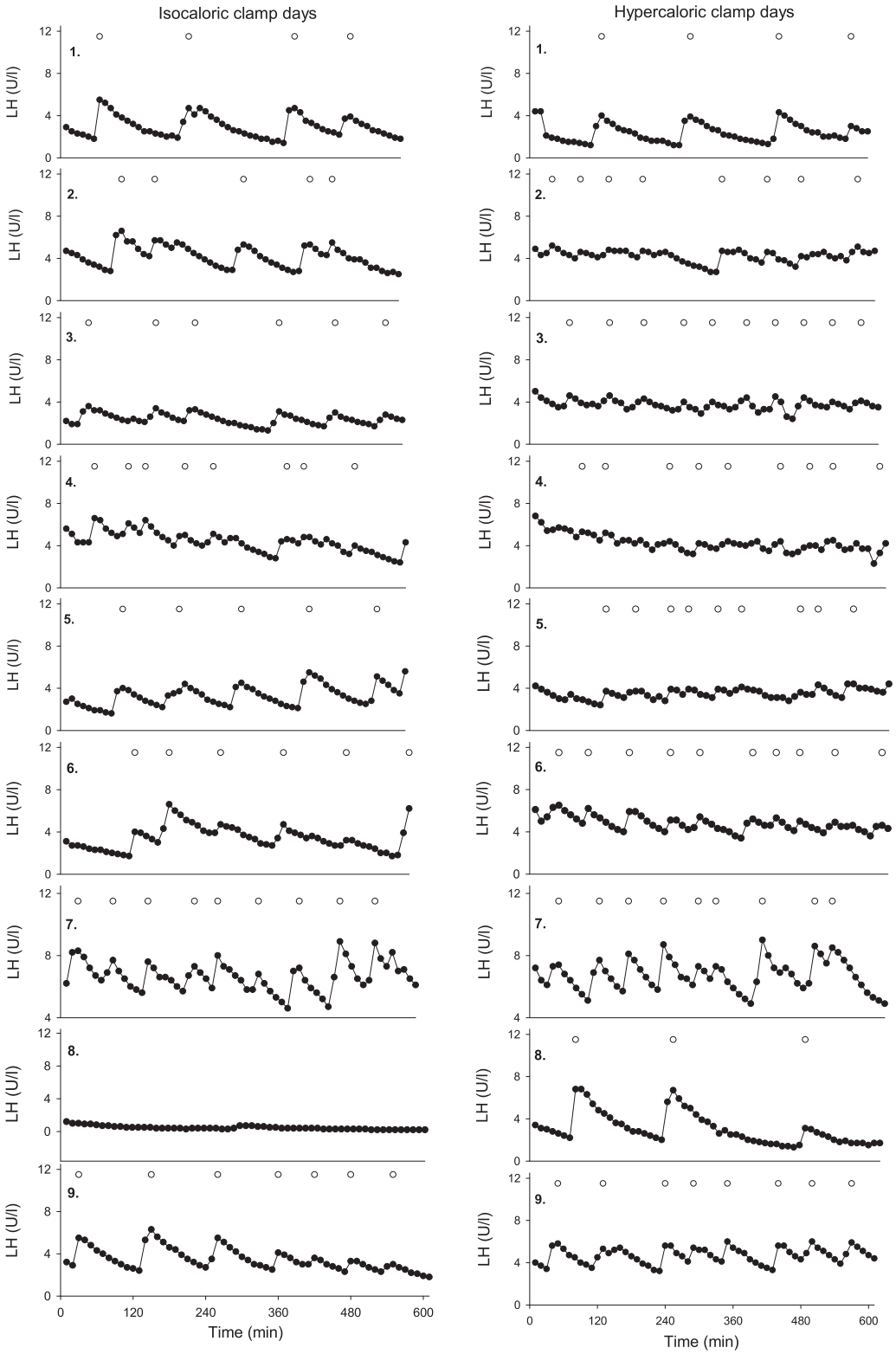

Figure 3. Individual profiles of insulin-stimulated LH secretion during HF (left) and HC (right). Open circles indicate significant peaks. HC, hypercaloric clamp day; HF, hypercaloric fasting day.

Figure 3 displays the insulin-stimulated LH secretion profiles of each volunteer before and after the hypercaloric feeding. Similar to that observed for spontaneous LH secretion, the LH peak frequency was increased in seven individuals at the end of the hypercaloric feeding, and remained unchanged for the remaining two subjects (number 1 and number 7). The mean LH pulse amplitude was decreased at the end of the hypercaloric feeding period in

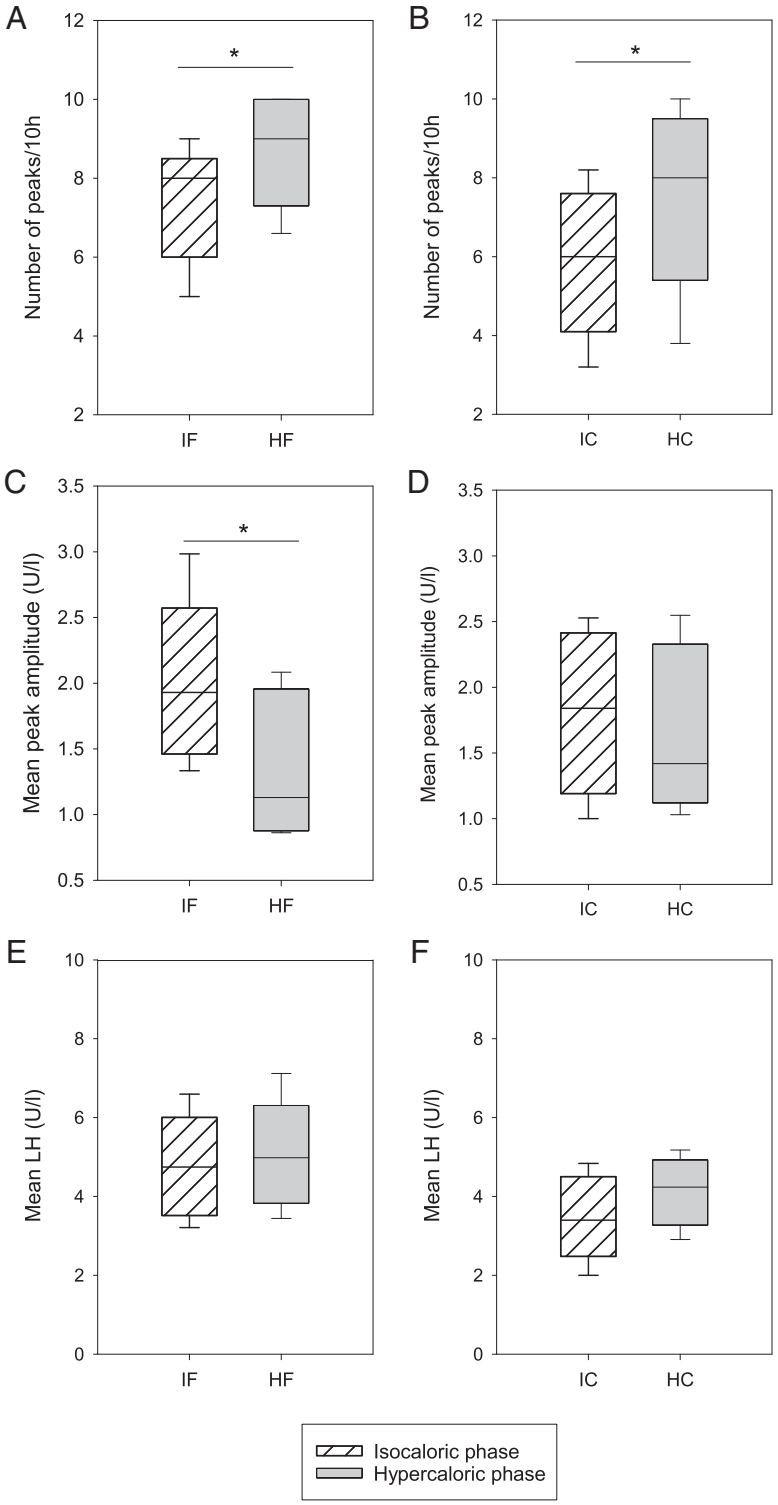

Figure 4. Characteristics of pulsatile LH secretion. A and B, LH peak frequency. C and D, LH peak amplitude. E and F, Mean LH levels. *, $P < .05$. HC, hypercaloric clamp day; HF, hypercaloric fasting day; IC, isocaloric clamp day; IF, isocaloric fasting day. Data are represented as median, 10th, 25th, 75th, and 90th percentiles.

seven of the nine volunteers. Exceptions were volunteers number 8 and number 9.

Figure 4 summarizes the changes occurring in pulsatile LH secretion. At the end of the hypercaloric diet, the spontaneous LH pulse frequency (Figure 4A) was increased by 17.9% ± 9.0% ($P \leq .05$), whereas the amplitude of the pulses (Figure 4C) was decreased by 26.5% ± 9.0% ($P \leq .05$). These changes in the LH pulse amplitude were positively correlated with the changes occurring in whole-body insulin sensitivity (Figure 5). The LH pulse frequency was also increased by 29.9% ± 10.2% during the insulin clamp performed at the end of the hypercaloric diet (Figure 4B, $P \leq .05$). Finally, the mean LH levels were not affected by the intervention.

Discussion

Obesity has been associated with alterations in neuroendocrine reproductive hormones that have been linked to impaired fertility (37), but little is known regarding the mechanisms implicated. To investigate the role of peripheral insulin resistance in this context, young and lean women with normal menstrual cycles were studied before and at the end of 1 month of administration of a hypercaloric diet. This high-sugar, high-fat diet, calculated to attain approximately 150% of their daily energy requirements, induced significant increases in body weight, decreases in whole-body insulin sensitivity, and increases in fasting insulin levels. Patterns of pulsatile LH secretion were significantly altered at the end of hypercaloric feeding, when compared with the isocaloric diet. Changes consisted of an increased frequency of pulsatile LH secretion with lower amplitude pulses, both in spontaneous and insulin-stimulated conditions. Of note, the respective direction of these changes is entirely coherent, with these two parameters of LH pulsatility being somewhat interdependent, adding to the robustness of our observations.

A modulation in the frequency of pulsatile LH secretion cannot occur at the pituitary level. Therefore, the increase

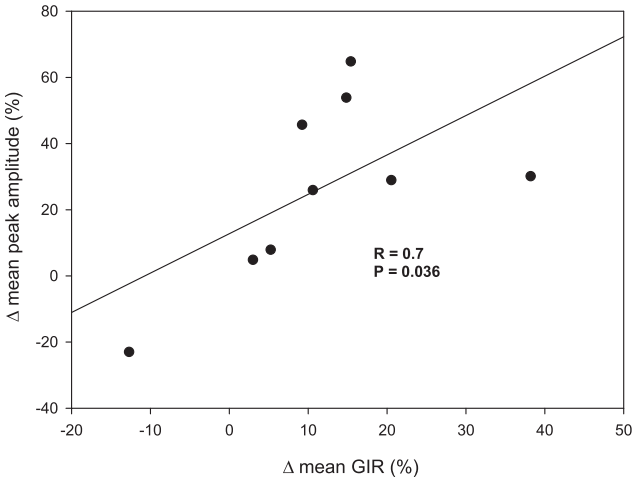

Figure 5. Positive correlation between LH pulse amplitude and whole-body insulin sensitivity. The value of r was obtained by a nonparametric Spearman's correlation test.

observed in LH pulse frequency is likely a reflection of a hypothalamic effect of our intervention (38, 39). Rodent data showing an acceleration of the frequency of pulsatile GnRH secretion from hypothalamic explants obtained from diet-induced obese mice suggest that this effect could be mediated by GnRH neurons (40). In contrast, the origin of the decrease in LH peak amplitude could be either at the hypothalamic or at the pituitary level (38). Indeed, a smaller peak amplitude could result from a stimulation by lower concentrations of GnRH, reflecting decreased hypothalamic GnRH secretion. However, it could equally result from a modification of the sensitivity of the pituitary gonadotroph cells to GnRH stimulation, as previously reported in another model of diet-induced obese mice (41). Although we found no overall effect of hypercaloric feeding on LH pulse amplitude during the insulin clamps, changes were observed at the individual level. The LH pulse amplitude was indeed decreased during the clamps performed at the end of the period of hypercaloric feeding in seven of the nine volunteers. The two exceptions were volunteer number 8, who displayed no peak at all during the isocaloric clamp test day, and volunteer number 9, who experienced an increase in insulin sensitivity at the end of the hypercaloric diet. Thus, the increase in the mean peak amplitude of volunteer number 9 is entirely consistent with the positive correlation observed between spontaneous LH pulse amplitude and whole-body insulin sensitivity.

These data confirm the existence of an important link between insulin and the control of LH secretion in humans (29), possibly related to the long-term elevations in peripheral insulin levels occurring in states of insulin resistance. Indeed, one can hypothesize that the decreases in whole-body insulin sensitivity observed at the end of hypercaloric feeding are not paralleled by similar decreases at the level of the CNS, rendering the hypothalamo-pituitary unit highly sensitive to these raised insulin levels. This hypothesis is entirely consistent with rodent data, in which alterations in the neuroendocrine reproductive hormones similar to those reported here are induced by changes in insulin signaling within GnRH neurons as well as pituitary gonadotroph cells (40, 41).

All the volunteers experienced weight gain at the end of the hypercaloric diet, resulting essentially from an increase in fat mass. Thus, our intervention induced modifications in body composition very similar to what is usually observed in more chronic conditions of excessive energy intake together with a number of changes in blood markers. In particular, fasting glycemia, fasting insulinemia, and the insulin resistance index HOMA-IR were all increased at the end of the hypercaloric diet. As stated above, whole-body insulin sensitivity measured during the hyperinsulinemic euglycemic clamp studies was also significantly decreased at the end of the hypercaloric diet, although it clearly did not reach values typically associated with insulin resistance such as those seen in type 2 diabetes mellitus.

These metabolic modifications are nevertheless physiologically significant, again reminiscent of the early stages of obesity. Interestingly in this context, decreases in LH pulse amplitude have been reported in obese women (42). The present data demonstrate that short-term hypercaloric feeding of normal volunteers can reproduce some of the neuroendocrine modifications associated with excess weight of much longer duration. They also demonstrate that these alterations appear early in states of excessive caloric intake in humans, during the phase of dynamic weight gain, validating further the use of this model in the study of human obesity.

As previously reported (43–45), adiponectin was higher at the end of the hypercaloric feeding period, confirming the existence of a differential regulation between short- and long-term conditions of positive energy balance (46, 47). We also observed a large rise in leptin levels after the hypercaloric feeding, consistent with the increase observed in fat mass. Given the well-known actions of leptin in both the regulation of energy intake and body weight and the neuroendocrine reproductive system, leptin was a logical candidate link between metabolic changes and the hypothalamic GnRH pulse generator in our study. However, we did not observe any significant correlation between parameters of LH secretion and leptin levels. Thus, the question of the implication of leptin in conveying signals of excessive energy intake to the hypothalamic GnRH pulse generator remains open.

In conclusion, we could demonstrate that 28 days of hypercaloric feeding in normal female volunteers can in-

duce alterations in the activity of the neuroendocrine reproductive axis that are reminiscent of the changes reported in more chronic states of obesity. Thus, our data suggest that these alterations may take place at very early stages of the disease. By showing changes in both the frequency and the amplitude of LH secretion pulses, our data also suggest that this modulation is occurring both at the hypothalamic and the pituitary levels. Moreover, the existence of a significant correlation between changes in whole-body insulin sensitivity and these hormonal alterations is providing for the first time a mechanistic explanation linking the neuroendocrine dysfunctions associated with calorie overload and obesity to insulin signaling. Further work in infertile obese patients will be necessary to better delineate the therapeutic implications of our observations.

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