Pulsatile gonadotropin-releasing hormone therapy in persistent amenorrheic weight-recovered anorexia nervosa patients

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Objective: To compare hormonal and clinical responses to GnRH pulsatile treatment in weight-recovered anorexia nervosa patients (Rec-AN) with persistent functional hypothalamic amenorrhea (HA) vs. in patients with secondary and primary HA.
Design: Retrospective, observational, ambulatory study.
Setting: University hospital.
Patient(s): Forty-one women: 19 Rec-AN (body mass index >18.5 kg/m^2 without menses recovery), 15 secondary HA without any eating disorders patients (SHA), and 7 primary HA patients (PHA).
Intervention(s): Gonadotropin-releasing hormone pulsatile therapy.
Main Outcome Measure(s): Baseline E_2, LH, and P plasma levels and their changes during induction cycles; ovulation, follicular recruitment, and pregnancies.
Results: The Rec-AN group displayed higher basal E_2 and LH plasma levels after GnRH injection compared with SHA and PHA. Higher E_2 and LH levels were observed during induction cycles in Rec-AN compared with SHA and PHA. Follicular recruitment was higher in Rec-AN. The ovulation rate was higher in Rec-AN compared with PHA but similar to SHA.
Conclusion(s): This study showed increased gonadal status and higher E_2 response to pulsatile GnRH therapy in persistent amenorrheic weight-recovered AN compared with HA from other causes. It suggests that their individual set-point of body weight allowing a fully functional gonadal axis is not reached yet. Specific factors of gonadal inertia in Rec-AN still remain unclear. (Fertil Steril® 2017;107: 502–9. ©2016 by American Society for Reproductive Medicine.)
Key Words: Anorexia nervosa, hypothalamic amenorrhea, pulsatile GnRH therapy

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Anorexia nervosa (AN) is an eating disorder affecting mainly women (1, 2). It is characterized by self-starvation leading to noninflammatory undernutrition and hormonal changes (3), such as blunted leptin plasma level, low insulin-like growth factor type 1 (IGF-1) plasma level, low free tri-iodothyronine (T3) syndrome, and high cortisol plasma level (4). Despite recent modification in the *Diagnostic and Statistical Manual of Mental Disorder* definition (5), AN is also associated with a functional hypothalamic amenorrhea in women, defined by the inability for the hypothalamus to deliver pulsatile GnRH secretion, leading to blunted functioning of the gonadotropic axis (4).

Menses resumption during the weight gain process is still relevant for nutritional recovery monitoring. Unfortunately, weight gain recovery above the lower limits of weight normality associated with normalization of the altered nutritional markers cited above, although indispensable, is

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not always sufficient to restore the gonadotropic function in AN patients (1). An individual set-point of body weight and body composition was indeed suggested (6), but the underlying causes of persistent amenorrhea despite full weight recovery still need to be explained and/or explored (7).

In clinical practice, fertility can be restored in hypothalamic amenorrhea patients using pulsatile GnRH therapy with a pump, to re-establish GnRH pulse (8–11). This medical device, installed either IV or SC, delivers pulsatile doses of GnRH every 90 minutes. Contrary to other assisted reproductive technologies, it replicates normal physiology by stimulating normal secretion of LH and FSH from the pituitary, allowing physiologic maturation of ovarian follicles. The efficacy and safety of this device has been tested in several studies in the past, with cumulative pregnancy rates between 70% and 93% after an average of six cycles of induction, pregnancy rate per cycle between 18% and 45%, and ovulation rate per cycle between 70% and 95% in hypothalamic amenorrhea patients (12–17). Unfortunately, underweight patients were frequently included in those studies, questioning the possible high number of weight nonrecovered AN patients using this assisted reproductive procedure. On the other hand, only a few retrospective studies have been conducted in AN patients. Small numbers and heterogeneity of the studied populations were the main limitations of these trials, mixing indeed sometimes weight-recovered and nonrecovered patients (12, 18, 19). The induction procedure was also different in terms of hormonal pretreatment and using different methods of luteal phase sustaining procedure, sometimes in the same retrospective study. To date, data on hormonal changes and fertility restoration with GnRH treatment in persistent amenorrheic weight-recovered AN are rare. In addition, comparison data on the hormonal response of this entity with primary and secondary hypothalamic amenorrhea not related to eating disorders is also lacking.

The primary purpose of our study was therefore to compare pituitary and gonadal responses to GnRH pulsatile treatment in three well-defined female groups with hypothalamic amenorrhea: [1] weight-recovered AN patients with persistent amenorrhea, [2] patients with secondary hypothalamic amenorrhea with no eating disorders, and [3] patients with primary hypothalamic amenorrhea of other causes. Comparison of the pregnancy success rate among the different groups as a clinical outcome was our secondary purpose.

MATERIALS AND METHODS

Ethics

This was a retrospective, observational, monocentric study. The local institutional research and ethics committee of Saint-Etienne, France approved the study. Data collection continued from August 2001 to March 2013.

Subjects

Forty-one female ambulatory patients with hypothalamic amenorrhea, seeking pregnancy and eligible for pulsatile GnRH therapy in our center, were included in this study (no LH pulse, evaluated by blood sampling every 10 minutes during 4 hours; no polycystic ovary syndrome [PCOS] [20]; strictly normal hysterosalpingogram and normal partner's semen evaluation, interpreted according to recommendations at the time they were performed).

Patients were divided among three groups according to the underlying cause of their hypothalamic amenorrhea, as follows.

Nineteen women with persistent amenorrheic weight-recovered anorexia nervosa (Rec-AN) were included. Weight-recovered was defined as body mass index >18.5 kg/m^2 and no biological markers of undernutrition (free T3, IGF-1, and cortisol). All patients had previous agreement of the psychiatrist in charge of their treatment.

Fifteen patients with primary hypothalamic amenorrhea (PHA), for whom menstruation never started, were also included. Eight of 15 patients tested positive for genetic Kallman syndrome.

Seven patients had secondary hypothalamic amenorrhea (SHA), defined as cessation of menstruation cycles for more than 6 months, but no eating disorder and undernutrition history. In five patients a psychological trauma preceding amenorrhea could be identified (one sexual harassment, two ended a relationship, and two aggressions).

Exclusion criteria were PCOS, tubal obstruction, endometriosis, undernutrition, and sperm abnormalities.

Infusion Pump Procedure

Gonadotropin-releasing hormone pulsatile therapy was administered using a portable SC infusion pump (Zyklomat, Ferring SA) containing 3.2 mg of GnRH (Lutreref, Ferring SA). Frequency and dose were set up according to previously published data (13): 20 μg every 90 minutes during 4 weeks.

No estroprogestative pretreatment was administered to any patient before the pulsatile GnRH therapy. The pump was then placed on the abdomen of the patient for delivering GnRH SC for 4 weeks. The device was maintained during all the induction cycle (defined as pulsatile GnRH therapy for 4 weeks), and no other hormonal treatment was added to trigger ovulation or to support luteal phase. Conception occurred upon intercourse.

Study Design

Each patient underwent the same procedure throughout the study.

At baseline visit, data were gathered on weight, height, and age, as well as from initial hormonal and nutritional assessment (leptin, free T3, IGF-1). Cortisol over 24 hours was recorded, to eliminate other endocrine diseases leading to hypothalamic amenorrhea. Baseline gonadotropin plasma levels (E_2, LH, and FSH) were also measured, as well as LH and FSH response 30 minutes after IV injection of 100 μg of GnRH. Luteinizing hormone pulse was evaluated by blood sampling every 20 minutes during 4 hours.

The SC infusion pump was then placed on the patient, who was educated in managing the device. Frequency and

doses were, as mentioned above, set up at 90 minutes during 4 weeks. The device was not removed until the end of the cycle.

The induction cycle was repeated with the same procedure if no pregnancy occurred.

During each induction cycle, blood samples were collected every 3 days to assay E_2, LH, and P plasma levels. Ambulatory ultrasound was performed every 3 days, and the number of ovarian follicles was recorded.

Adverse effects (multiple pregnancies; clinical ovarian hyperstimulation syndrome, defined as abdominal pain, nausea, abdominal effusion, and increased ovarian volume [21]; pain or infection at the puncture site; and reactivation of eating disorder) were recorded regularly.

The threshold of 5 ng/mL of P was considered to define the occurrence of ovulation (22). Pregnancy was considered as valid after the birth of a living baby.

Blood Sampling and Ultrasound Evaluation

Estradiol (ng/L), FSH (U/L), free T3 (pmol/L), cortisol (nmol/L), and LH (U/L) were assayed by Cobas 8000 (Roche Laboratories); IGF-1 (μg/L) by YSIS (IDS) and leptin (ng/mL) by immunoenzymatic assay and ELISA. IGF-1 (ug/L) were assayed by electrochemiluminescence on COBAS PLC. Luteinizing hormone (U/L) was assayed by electrochemiluminescence on ABBOTT and leptin (ng/mL) by immunoenzymatic assay and ELISA. Follicular recruitment was considered effective when the presence of a follicle >14 mm on ultrasound was observed.

Follow-up assay and ultrasound were performed in the ambulatory position by a single operator for each patient.

Statistical Analysis

All values are presented as mean ± SEM. A nonparametric Mann-Whitney test was used to compare baseline parameters, number of pregnancies, ovulation rate, and follicular recruitment between groups. A two-factor repeated-measures analysis of variance followed by post hoc test was used to compare LH and FSH values in the pulse tests and changes in E_2 and LH plasma levels during cycles of induction between the groups. Because conception occurred upon spontaneous intercourse, all cycles with and without ovulation and fecundation were pooled in statistics analysis and figures. Statistical significance was set at $P<.05$. All statistical analyses were performed with StatView 4.5 software (Abacus Concepts).

RESULTS

Baseline Characteristics

Weight and body mass index was significantly decreased in Rec-AN compared with SHA ($P=.0001$ and $P=.0001$, respectively). However, no differences were observed for leptin, free T3, cortisol, ACTH, and IGF-1 between the groups (Table 1).

Gonadotropic initial assessment showed that E_2 was significantly increased in Rec-AN compared with PHA ($P=.0001$) and SHA ($P=.001$). Follicle-stimulating hormone was also significantly higher in Rec-AN than in PHA ($P=.009$) (Table 1). Analysis of variance of pulse evaluation of LH plasma level showed no change in values during the 4-hour sampling in all groups.

TABLE 1

Baseline characteristics.

Characteristic	Rec-AN (n = 19)	SHA (n = 15)	PHA (n = 7)
Age (y)	27.8 ± 0.8	29.1 ± 1.3	25.3 ± 1.4
Weight (kg)	50.7 ± 2.3	57.6 ± 2.7	72.4 ± 4.6[a,b]
Height (m)	1.63 ± 0.12	1.65 ± 0.02	1.64 ± 0.02
Body mass index (kg/m^2)	19.1 ± 0.6	21.3 ± 1.1	26.8 ± 1.3[a]
Leptin (ng/L)	8.1 ± 2.2	14.1 ± 7.2	8.4 ± 2.5
Free T3 (pmol/L)	3.4 ± 0.4	5.1 ± 1.2	4.0 ± 0.3
24-h mean cortisol (nmol/L)	136.6 ± 53.4	164.2 ± 95.7	156.6 ± 19.6
IGF-1 (μg/L)	193.5 ± 24.1	208.1 ± 37.6	177.5 ± 34.6
E_2 (ng/L)	21.2 ± 2.6	12.3 ± 1.8[a]	13.6 ± 3.2[a]
FSH (U/L)	5.8 ± 0.6	5.5 ± 0.8	1.6 ± 0.6[a,b]
LH (U/L)	2.3 ± 0.5	2.8 ± 0.6	1.2 ± 0.8

Note: Data are expressed as mean ± SEM.
[a] $P<.05$ vs. Rec-AN.
[b] $P<.05$ vs. SHA.

Germain. GnRH pulsatile therapy in anorexia nervosa. Fertil Steril 2016.

After the injection of 100 μg of GnRH, LH plasma levels significantly increased at 30 minutes in all groups ($P=.001$ in Rec-AN, $P=.03$ in PHA, and $P=.0016$ in SHA) (Fig. 1A and B). The LH plasma level after GnRH injection was also significantly higher in Rec-AN (27.7 ± 4.9 U/L) than in PHA (14.3 ± 4.4 U/L, $P=.05$) and SHA (10.8 ± 2.6 U/L, $P=.008$) (Fig. 1A). The FSH plasma level also significantly increased 30 minutes after the injection of 100 μg of GnRH ($P=.0016$ in Rec-AN, $P=.02$ in PHA, and $P=.0013$ in SHA), without any difference between the groups (Fig. 1B).

Clinical Monitoring of Inductions Cycles

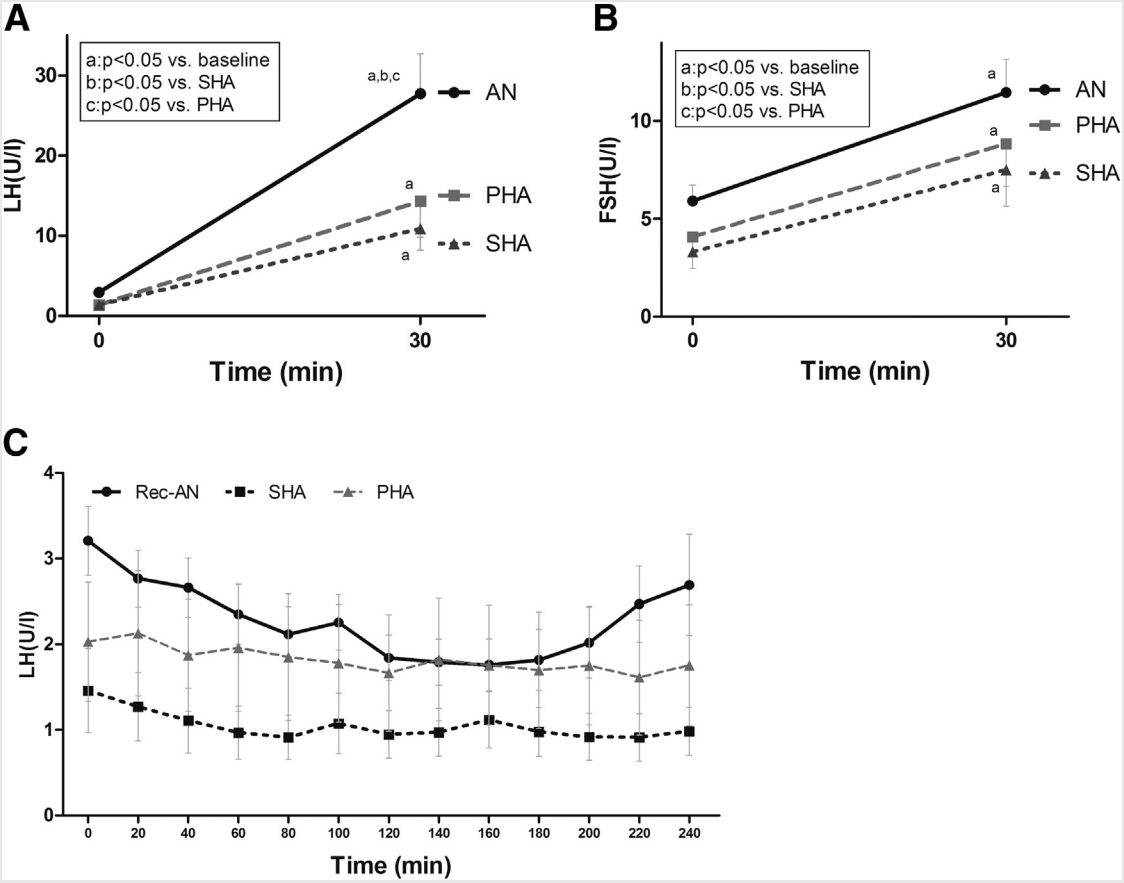
A total of 146 inductions cycles were analyzed (Table 2). Sixty-four inductions were performed in the Rec-AN group. Four of them underwent two sets of induction at different moments of their lifetime. Forty-nine inductions were performed in the SHA group. Three SHA patients had two sets of induction cycles at different moments of their lifetime. Finally, 33 inductions were performed in the PHA group. Two PHA patients had two sets of induction cycles at different moments of their lifetime. The average number of inductions per set was significantly higher in PHA compared with SHA ($P=.03$) but similar to Rec-AN.

Follicular recruitment was higher in Rec-AN compared with SHA ($P=.02$) and PHA ($P=.006$). No differences were observed between PHA and SHA ($P=.5$).

Twenty-six pregnancies were observed (positive fetal heartbeat) during the study period: 14 in Rec-AN patients for 12 live births, 11 in the SHA group for 8 live births, and 1 in the PHA group for 1 live birth. The cumulative pregnancy rate (positive fetal heartbeat) for Rec-AN was 74% (63% for live birth), 73% (53% for live birth), and 14% (14% for live birth) for PHA ($P=.03$ and $P=.01$ in PHA vs. SHA and Rec-AN, respectively).

Five miscarriages were observed, two in the Rec-AN group and three in the PHA group. One twin pregnancy was noted in a twin family in the Rec-AN group. No adverse event

FIGURE 1



Basal gonadotropic assessment in the three groups of the study. Basal GnRH test results for (**A**) LH and (**B**) FSH. Luteinizing hormone and FSH plasma level response after IV injection of 100 μg of GnRH. (**C**) Luteinizing hormone plasma level pulse assessed by every 20 minutes samplings for 3 hours.

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related to the catheter was recorded. No episode of ovarian hyperstimulation and one episode of excessive response occurred. It is of note that re-emergence of an eating disorder occurred in three Rec-AN patients. One happened during pregnancy with bulimic crisis and two after delivery with restrictive eating behavior, one immediately after delivery and the other in less than 6 months.

None of the patients recovered gonadal functioning after the induction cycle and/or after delivery.

Hormonal Monitoring of Induction Cycles

Ovulation rate, based on P plasma level, was similar between Rec-AN and SHA (84% ± 0.5% in Rec-AN and 78% ± 0.7 % in SHA, $P=.5$ vs. Rec-AN) but lower in PHA (36% ± 1.0 %, $P=.0001$ vs. Rec-AN and $P=.001$ vs. SHA) (Table 2).

Analysis of E_2 plasma level changes over inductions is presented in Figure 2A. Estradiol increased physiologically during the follicular phase and decreased during the luteal phase in the three groups. In Rec-AN the E_2 plasma level increased significantly higher than in the other groups from day 3 to day 21, with a maximum peak after 10 days of GnRH pump treatment (326.8 ± 50.8 ng/L in Rec-AN; 142.2 ± 23.7 ng/L in SHA, $P=.009$ vs. Rec-AN; 71.2 ± 21.6 ng/L in PHA, $P=.001$ vs. Rec-AN). The mean plasma level of E_2 in Rec-AN was significantly elevated compared with the other groups (210.9 ± 26.9 ng/mL in Rec-AN; 110.9 ± 13.2 ng/L in SHA, $P=.0032$ vs. Rec-AN; 57.3 ± 10.5 ng/L in PHA, $P=.0002$ vs. Rec-AN). The mean plasma level of E_2 was significantly higher in SHA than in PHA ($P=.0053$).

Analysis of LH plasma level changes over the inductions is presented in Figure 2B. Luteinizing hormone plasma levels increased in the follicular phase and decreased in the luteal phase in the three groups. The LH plasma level peak was significantly higher in Rec-AN (13.1 ± 2.1 U/L) compared with PHA (6.2 ± 2.5, $P=.03$) but similar to SHA (10.4 ± 3.7, $P=.4$). Interestingly LH peaked later in Rec-AN than in SHA and PHA (after 5 days vs. 3 days of treatment). The mean LH plasma level during induction cycles was significantly higher in Rec-AN (8.01 ± 0.74 U/L) than in PHA (4.2 ± 0.39 U/L, $P=.0013$) but similar to SHA (6.9 ± 0.64 U/L, $P=.27$). The mean LH plasma level was higher in SHA than in PHA ($P=.0035$).

TABLE 2

Clinical monitoring of inductions.

Variable	Rec-AN (n = 19)	SHA (n = 15)	PHA (n = 7)
No. of patients	19	15	7
No. of induction cycles	64	49	33
No. of induction cycles per set (min-max)	2.5 ± 0.2 (1–8)	2.1 ± 0.1 (1–5)	2.8 ± 0.3 (1–8)[b]
Percent ovulation rate	84 ± 0.5	78 ± 0.7[a]	36 ± 1.0[a,b]
Total no. of clinical pregnancies (positive fetal heartbeat/live birth)	14/12	11[a]/8[a]	1[a,b]/1[a,b]
Percent cumulative pregnancy rate (positive fetal heartbeat/live birth)	74/63	73/53	14/14[a,b]
Percent maximum follicular recruitment rate	100 ± 0.0	88.2 ± 5.6[a]	82.1 ± 7.4[a,b]
Eating disorder reactivation, n	3	NA	NA
Excessive response, n	1	0	0

Note: Data are expressed as mean ± SEM unless otherwise noted.
[a] $P<.05$ vs. Rec-AN.
[b] $P<.05$ vs. SHA.

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DISCUSSION

This study is the first to show data on well-phenotyped hypothalamic amenorrhea patients.

The main result of this study is the discrepancy between the groups in hormonal and clinical monitoring of the induction cycle. Indeed, weight-recovered AN exhibited a higher baseline E_2 plasma level compared with the other groups. Moreover, the LH response to GnRH injection was higher in weight-recovered AN compared with the other groups. These data suggest a higher baseline gonadal tone in weight-recovered AN than in hypothalamic amenorrhea from other causes. Those data are strengthened by hormonal changes during induction cycles showing higher E_2 all along the induction cycle and a higher LH peak, as well as a greater follicular recruitment and higher ovulation rate. This is in line with a study published in 1986 showing a high ovulation rate in AN patients (19). All those data suggest that the remaining blunted gonadal axis seems to be less pronounced and/or more prone to be stimulated in those persistent amenorrheic weight-recovered AN than in the non-nutritional hypothalamic amenorrheic patients, even if none of the patients recovered functioning gonadal axis after delivery.

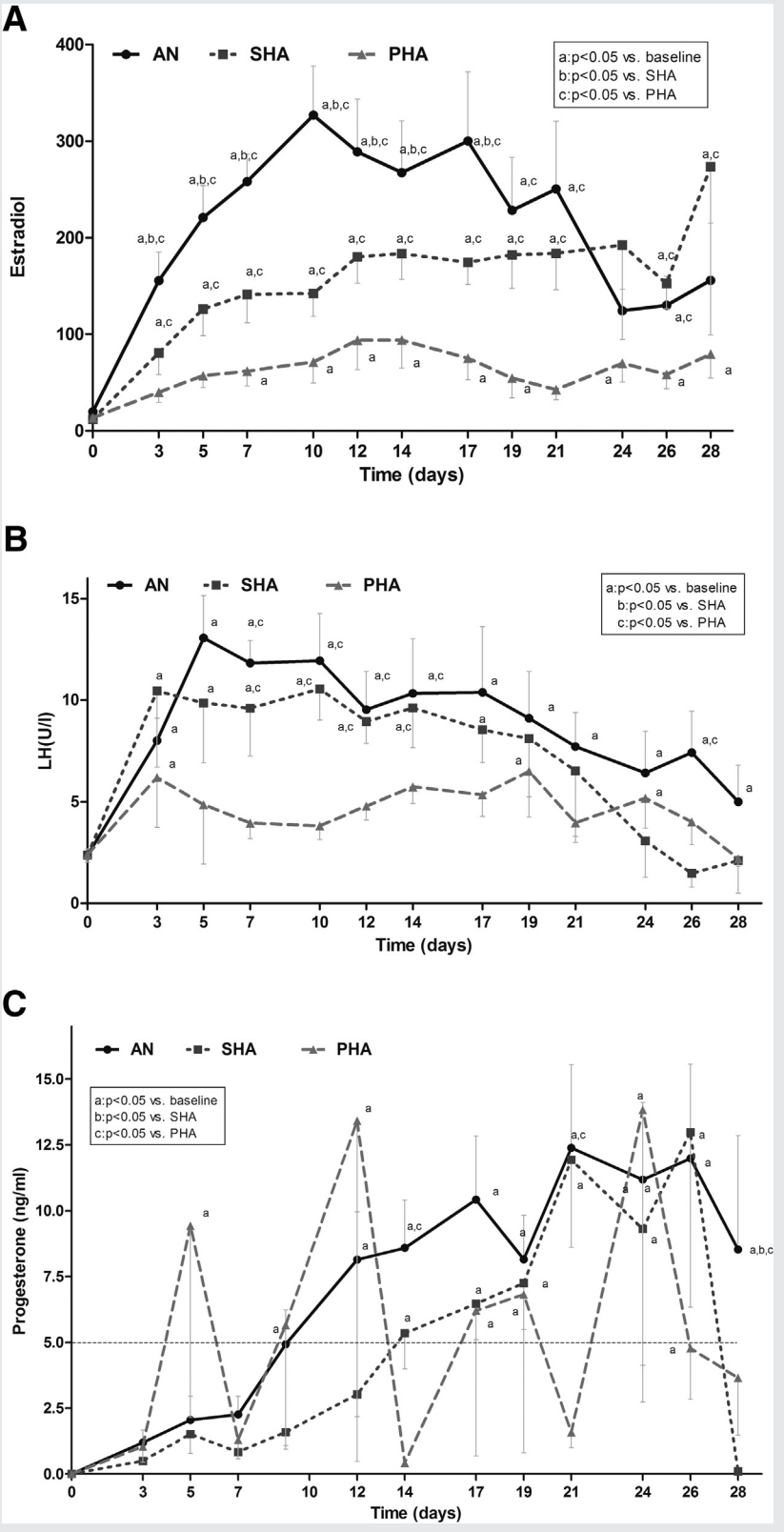
This "over ability" to be stimulated in weight-recovered AN is surprising because those patients are thinner than the other patients, even if they recovered a body mass index above 18.5 kg/m^2 and displayed no significant obvious biological markers of undernutrition. This could be explained by a rebound effect of the gonadal axis after being blunted by undernutrition: it has been shown in several studies that LH response could increase after weight recovery and could be higher than in controls (23–25).

This could explain why those weight-recovered anorexia nervosa patients do not recover their menses despite body weight gain resumption, suggesting that the individual set-point of body weight allowing a fully functional gonadal axis is not reached yet in those patients. In underweight AN indeed, loss of LH pulse is explained by a decreased fat mass associated with blunted leptin plasma level and lack of kisspeptin (26), hypercortisolism, and growth hormone axis abnormalities (27). Adaptive high ghrelin level is also able to blunt LH pulse as part of the multiple causes of the hypothalamic amenorrhea found in AN (28). Moreover, opioid and endorphin excesses due to high stress levels are also able to block the gonadotropic axis (29, 30). Finally excess physical activity, frequently seen in AN, leads to both opioid excess and a relative decrease in fat mass due to increase in muscle/free fat mass. These abnormalities contribute to perpetuating hypothalamic amenorrhea (28, 29, 31). Those latter parameters were not evaluated in this study. Recently, injection of recombinant kisspeptin or naloxone has been shown to restore LH pulse (32–35). Finally, the "over ability" to be stimulated by pulsatile GnRH therapy suggests that the gonadal axis in persistent amenorrheic weight-recovered AN women is functional and that little should be needed to trigger it on. It would be of interest to fully explore those recovered AN patients with regard to their hormonal/nutritional status. However, pulsatile secretion of GnRH is dependent on many factors, some of which cannot be explored in vivo.

This study also showed the difference in hormonal change between primary and secondary hypothalamic amenorrhea, with higher response in secondary hypothalamic amenorrhea. Even if these data need to be analyzed with caution with regard to the low number of patients in each group, it is in line with a recent study showing that primary genetic hypothalamic amenorrhea was less responsive than controls to GnRH pulsatile therapy (36), even if it remains one of the recommended treatments (11). It would be of interest to differentiate organic from functional etiologies, to better understand those discrepancies between the groups.

This retrospective study shows a global efficacy and tolerability of pulsatile GnRH therapy in persistent amenorrhea weight-recovered AN patients, with a cumulative rate of birth with living babies of 63%. This lower rate compared with previous published studies is explained by the fact that we included in the statistical analysis only the pregnancy leading to a living baby and excluded miscarriages (22). Besides, this efficacy was obtained without luteal-phase sustaining treatment or triggering ovulation, as is sometimes proposed (18). No complication occurred during all these simple procedures, with well-phenotyped patients in whom PCOS was excluded. Pulsatile GnRH therapy has been shown to be more effective

FIGURE 2



Hormonal monitoring of inductions. (**A**) Estradiol, (**B**) LH, and (**C**) P changes during induction in the three groups of the study. All data were included in figures and statistics analysis whether or not there were ovulation and fecundation.

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than exogenous gonadotropins therapy, but patients were less phenotyped than in our study (37); no study has compared so far pulsatile GnRH therapy and exogenous gonadotrophins in recovered AN. However, our data along with literature could suggest that pulsatile GnRH therapy is a good choice of treatment in hypothalamic amenorrhea, without side effects.

Finally, the reactivation of three cases of eating disorder in our study leads us to wonder whether medicine should always absolutely meet the desire of pregnancy of AN patients, despite their fragile psychological and nutritional conditions. It thus seems necessary to check whether this intervention contributes to the cure of the disease or its recurrence. It seems important to be able to properly and carefully check on both nutritional and psychiatric status before starting pulsatile GnRH therapy in a multidisciplinary consultation meeting, considering the possible adverse consequences of the reactivation of such a disorder on the course of pregnancy (38) and the health of the unborn child in terms of low birth weight or prematurity (39, 40). This beforehand checking seems critical with regard to frequent underweight patients included in GnRH published series.

This study could raise some limitations. The small number of patients is counteracted by the high phenotyping of the groups. The ambulatory setting of this real-life study was highly monitored in the same place and by the same operator for each patient. Most importantly, our pulsatile GnRH therapy procedure was reproductive for all patients and shows significant discrepancies between well-phenotyped groups.

In conclusion, this study confirmed that pulsatile GnRH therapy is safe and efficient treatment in hypothalamic amenorrhea. It mainly showed both increased gonadal status and higher E_2 response to pulsatile GnRH therapy in persistent amenorrheic weight-recovered AN patients than in the other causes of hypothalamic amenorrhea. This could suggest that their individual set-point of body weight allowing a fully functional gonadal axis is not reached yet. Fully and comprehensive evaluation of hormonal complications of undernutrition may be helpful to phenotype patients who need to improve their nutritional status even if the weight is recovered.

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REFERENCES

1. Viricel J, Bossu C, Galusca B, Kadem M, Germain N, Nicolau A, et al. [Retrospective study of anorexia nervosa: reduced mortality and stable recovery rates]. Presse Med 2005;34:1505–10.
2. Revised diagnostic subgroupings for anorexia nervosa. Nutr Rev 1994;52:213–5.
3. Germain N, Galusca B, Le Roux CW, Bossu C, Ghatei MA, Lang F, et al. Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin. Am J Clin Nutr 2007;85:967–71.
4. Estour B, Germain N, Diconne E, Frere D, Cottet-Emard JM, Carrot G, et al. Hormonal profile heterogeneity and short-term physical risk in restrictive anorexia nervosa. J Clin Endocrinol Metab 2010;95:2203–10.
5. Attia E, Becker AE, Bryant-Waugh R, Hoek HW, Kreipe RE, Marcus MD, et al. Feeding and eating disorders in DSM-5. Am J Psychiatry 2013;170:1237–9.
6. Golden NH, Jacobson MS, Schebendach J, Solanto MV, Hertz SM, Shenker IR. Resumption of menses in anorexia nervosa. Arch Pediatr Adolesc Med 1997;151:16–21.
7. Jacoangeli F, Masala S, Staar Mezzasalma F, Fiori R, Martinetti A, Ficoneri C, et al. Amenorrhea after weight recover in anorexia nervosa: role of body composition and endocrine abnormalities. Eat Weight Disord 2006;11:e20–6.
8. Jaramillo C, Charro-Salgado A, Perez-Infante V, del Campo GL, Botella-Llusia J, Coy D, et al. Clinical studies with d-Trp 6-luteinizing hormone-releasing hormone in anovulatory women. Fertil Steril 1978;29:418–23.
9. Leyendecker G, Wildt L, Hansmann M. Pregnancies following chronic intermittent (pulsatile) administration of Gn-RH by means of a portable pump ("Zyklomat")—a new approach to the treatment of infertility in hypothalamic amenorrhea. J Clin Endocrinol Metab 1980;51:1214–6.
10. Leyendecker G, Wildt L. Pulsatile therapy of hypothalamic amenorrhea with gonadotropin releasing hormone. Dtsche Med Wochenschr 1984;109:462.
11. Boehm U, Bouloux PM, Dattani MT, De Roux N, Dodé C, Dunkel L, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. Nat Rev Endocrinol 2015;11:547–64.
12. Homburg R, Eshel A, Armar N, Tucker M, Mason P, Adams J, et al. One hundred pregnancies after treatment with pulsatile luteinising hormone releasing hormone to induce ovulation. BMJ 1989;298:809–12.
13. Braat D, Schoemaker R, Schoemaker J. Life table analysis of fecundity in intravenously gonadotropin-releasing hormone-treated patients with normogonadotropic and hypogonadotropic amenorrhea. Fertil Steril 1991;55:266–71.
14. Skarln G, Ahlgren M. Pulsatile gonadotropin releasing hormone (GnRH)—treatment for hypothalamic amenorrhoea causing infertility. Acta Obstet Gynecol Scand 1994;73:482–5.
15. Kesrouani A, Abdallah MA, Attieh E, Abboud J, Atallah D, Makhoul C. Gonadotropin-releasing hormone for infertility in women with primary hypothalamic amenorrhea. Toward a more-interventional approach. J Reprod Med 2001;46:23–8.
16. Filicori M, Flamigni C, Dellai P, Cognigni G, Michelacci L, Arnone R, et al. Treatment of anovulation with pulsatile gonadotropin-releasing hormone: prognostic factors and clinical results in 600 cycles. J Clin Endocrinol Metab 1994;79:1215–20.
17. Christin-Maitre S, de Crecy M. [Pregnancy outcomes following pulsatile GnRH treatment: results of a large multicenter retrospective study]. J Gynecol Obstetr Biol Reprod 2007;36:8–12.
18. Nillius SJ, Fries H, Wide L. Successful induction of follicular maturation and ovulation by prolonged treatment with LH-releasing hormone in women with anorexia nervosa. Am J Obstet Gynecol 1975;122:921–8.
19. Bringer J, Hedon B, Jaffiol C, Nicolau S, Gilbert F, Cristol P, et al. Influence of the frequency of gonadotropin-releasing hormone (GnRH) administration on ovulatory responses in women with anovulation. Obstet Gynecol Surv 1986;41:246–8.
20. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
21. Hull ME, Moghissi KS, Magyar DM, Hayes MF, Zador I, Olson JM. Correlation of serum estradiol levels and ultrasound monitoring to assess follicular maturation. Fertil Steril 1986;46:42–5.
22. Gronier H, Peigné M, Catteau-Jonard S, Dewailly D, Robin G. Induction de l'ovulation par administration pulsatile de GnRH en 2014: revue de la littérature et synthèse des pratiques courantes. Gynecol Obstet Fertil 2014;42:732–40.
23. Sherman BM, Halmi KA, Zamudio R. LH and FSH response to gonadotropin-releasing hormone in anorexia nervosa: effect of nutritional rehabilitation 1. J Clin Endocrinol Metab 1975;41:135–42.
24. Waldhauser F, Toifl K, Spona J, Frisch H. [Basal gonadotropin level and gonadotropin response to stimulation in patients with anorexia nervosa]. Wien Klin Wochenschr 1986;98:393–7.

25. Tomova A, Makker K, Kirilov G, Agarwal A, Kumanov P. Disturbances in gonadal axis in women with anorexia nervosa. Eat Weight Disord 2007;12:e92–7.
26. Holtkamp K, Mika C, Grzella I, Heer M, Pak H, Hebebrand J, et al. Reproductive function during weight gain in anorexia nervosa. Leptin represents a metabolic gate to gonadotropin secretion. J Neural Transm (Vienna) 2003; 110:427–35.
27. Roa J, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. New frontiers in kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function. Front Neuroendocrinol 2008;29:48–69.
28. Kluge M, Schussler P, Uhr M, Yassouridis A, Steiger A. Ghrelin suppresses secretion of luteinizing hormone in humans. J Clin Endocrinol Metab 2007;92:3202–5.
29. Ferin M. Clinical review 105: stress and the reproductive cycle. J Clin Endocrinol Metab 1999;84:1768–74.
30. Galusca B, Sigaud T, Costes N, Redoute J, Massoubre C, Estour B. Wide impairment of cerebral serotoninergic activity but inter-individual heterogeneity in bulimia nervosa patients: a pilot [(18)F]MPPF/PET study. World J Biol Psychiatry 2014;15:599–608.
31. Christo K, Cord J, Mendes N, Miller KK, Goldstein MA, Klibanski A, et al. Acylated ghrelin and leptin in adolescent athletes with amenorrhea, eumenorrheic athletes and controls: a cross-sectional study. Clin Endocrinol 2008; 69:628–33.
32. Jayasena CN, Abbara A, Veldhuis JD, Comninos AN, Ratnasabapathy R, De Silva A, et al. Increasing LH pulsatility in women with hypothalamic amenorrhoea using intravenous infusion of Kisspeptin-54. J Clin Endocrinol Metab 2014;99:E953–61.
33. Armeanu M, Berkhout G, Schoemaker J. Pulsatile luteinizing hormone secretion in hypothalamic amenorrhea, anorexia nervosa, and polycystic ovarian disease during naltrexone treatment. Fertil Steril 1992; 57:762–70.
34. Marrazzi M, Bacon J, Kinzie J, Luby E. Naltrexone use in the treatment of anorexia nervosa and bulimia nervosa. Int Clin Psychopharmacol 1995;10: 163–72.
35. Mendelson SD. Treatment of anorexia nervosa with tramadol. Am J Psychiatry 2001;158:963–4.
36. Abel BS, Shaw ND, Brown JM, Adams JM, Alati T, Martin KA, et al. Responsiveness to a physiological regimen of GnRH therapy and relation to genotype in women with isolated hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2013;98:E206–16.
37. Martin KA, Hall JE, Adams JM, Crowley WF Jr. Comparison of exogenous gonadotropins and pulsatile gonadotropin-releasing hormone for induction of ovulation in hypogonadotropic amenorrhea. J Clin Endocrinol Metab 1993;77:125–9.
38. Brinch M, Isager T, Tolstrup K. Anorexia nervosa and motherhood: reproduction pattern and mothering behavior of 50 women. Acta Psychiatr Scand 1988;77:611–7.
39. Franko DL, Blais MA, Becker AE, Delinsky SS, Greenwood DN, Flores AT, et al. Pregnancy complications and neonatal outcomes in women with eating disorders. Am J Psychiatry 2001;158:1461–6.
40. Linna MS, Raevuori A, Haukka J, Suvisaari JM, Suokas JT, Gissler M. Pregnancy, obstetric, and perinatal health outcomes in eating disorders. Am J Obstet Gynecol 2014;211:392.e1–8.