Estradiol levels in men with congenital hypogonadotropic hypogonadism and the effects of different modalities of hormonal treatment

Séverine Trabado, D.Pharm., Ph.D.,[a,b,c] *Luigi Maione, M.D.,*[a,c,d] *Sylvie Salenave, M.D.,*[a,e] *Stéphanie Baron, D.Pharm.,*[b] *Françoise Galland, M.D., Ph.D.,*[a,e] *Hélène Bry-Gauillard, M.D.,*[e] *Anne Guiochon-Mantel, M.D., Ph.D.,*[a,b,c] *Philippe Chanson, M.D.,*[a,c,e] *Nelly Pitteloud, M.D.,*[f] *Antonio Agostino Sinisi, M.D.,*[d] *Sylvie Brailly-Tabard, M.D., Ph.D.,*[a,b,c] *and Jacques Young, M.D., Ph.D.*[a,c,e]

[a] Paris-Sud University, Faculté de Médecine Paris-Sud, [b] Laboratoire de Génétique Moléculaire, Pharmacogénétique et Hormonologie, Assistance Publique-Hôpitaux de Paris, Hôpital de Bicêtre, and [c] INSERM U693, Le Kremlin-Bicêtre, France; [d] Endocrinology and Medical Andrology Section (LM, AS), Dipartimento Medico-Chirurgico di Internistica Clinica e Sperimentale "Magrassi-Lanzara," Seconda Universita' degli Studi di Napoli, Italy; [e] Service d'Endocrinologie et des Maladies de la Reproduction and Centre de Référence des Maladies Endocriniennes Rares de la Croissance, Assistance Publique-Hôpitaux de Paris, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France; and [f] Service d'Endocrinologie, Diabétologie et Métabolisme, Centre Hospitalier Universitaire Vaudois, Université de Lausanne, Lausanne, Switzerland

Objective: To evaluate the degree of E_2 deficiency in male congenital hypogonadotropic hypogonadism (CHH), and its response to different hormonal treatments.
Design: Retrospective and prospective studies.
Setting: Academic institution.
Patient(s): Untreated or treated CHH, healthy men, untreated men with Klinefelter syndrome (KS).
Intervention(s): Serum sex hormone-binding globulin (SHBG) and total E_2 (TE2) as well as bioavailable (BE2) and free (FE2) levels were measured and determined.
Main Outcome Measure(s): Total, bioavailable, and free testosterone, TE2, BE2, FE2 were compared in normal men, untreated and treated CHH and in untreated KS.
Result(s): TE2, BE2, and FE2 levels were very significantly lower in untreated patients with CHH (n = 91) than in controls (n = 63) and in patients with KS (n = 45). The TE2 correlated positively with serum total T in patients with CHH. The TE2 also correlated very positively with serum LH in the combined population of patients with CHH and healthy men, suggesting that low E_2 levels in CHH are due to severe LH-driven T deficiency. All fractions of circulating E_2 were very significantly higher in patients with CHH receiving T enanthate (n = 101) or the FSH–hCG combination (n = 88) than in untreated patients with CHH. Contrary to dihydrotestosterone (DHT), both T enanthate and combined FSH-hCG therapy significantly and prospectively increased TE2 levels in patients with CHH.
Conclusion(s): Contrary to KS, the male hypogonadism observed in CHH is associated with profound E_2 deficiency, which can be overcome by aromatizable androgen or combined gonadotropin therapy. (Fertil Steril® 2011;95:2324–29. ©2011 by American Society for Reproductive Medicine.)

Key Words: Estradiol, androgens, testosterone, hypogonadotropic hypogonadism, Kallmann syndrome, Klinefelter syndrome, osteoporosis

Congenital hypogonatropic hypogonadism (CHH) is a rare cause of severe T deficiency in young men (1, 2). It is also associated with abnormal bone development, no teenage growth spurt, and osteopenia or osteoporosis (1–5). Testosterone therapy has been shown to increase bone density in few of these patients, indicating a causal relationship between T deficiency and impaired acquisition and/or loss of bone mineral mass (4, 5). The effect of T on target tissues can be mediated either directly (by androgen receptors), or indirectly through E_2 produced by testicular T aromatization in Leydig cells or extragonadal T conversion into estrogens (E) by extragonadal aromatase (6). Thus, although androgens play a role in male skeletal health, their primary reason for being is increasingly challenged by direct and indirect evidence that E_2 also play a major role. Indeed, reports that altered E_2 production (aromatase loss-of-function mutations) and responsiveness (E_2 receptor $\tilde{\alpha}$ inactivating mutations) are associated with adverse skeletal effects in men strongly suggest that Es are critically important for male skeletal development and bone mineral density acquisition (6–8). The aim of this study was to evaluate in detail circulating E_2 levels in a series of young men with CHH to identify a possible

Received January 24, 2011; revised March 28, 2011; accepted March 29, 2011; published online May 4, 2011.
S.T. has nothing to disclose. L.M. has nothing to disclose. S.S. has nothing to disclose. S.B. has nothing to disclose. F.G. has nothing to disclose. H.B.-G. has nothing to disclose. A.G.-M. has nothing to disclose. P.C. has nothing to disclose. N.P. has nothing to disclose. A.A.S. has nothing to disclose. S.B.-T. has nothing to disclose. J.Y. has nothing to disclose.
Séverine Trabado and Luigi Maione contributed equally to this work.
Supported in part by grants from Paris Sud University (Bonus Qualité Recherche), Agence Nationale de la Recherche (ANR-KALGENOPATH 2009), Programme Hospitalier de Recherche Clinique "Hypoprotéo" (PHRC-2009), and from "Fondation pour la Recherche Médicale".
Presented at the XXVII Congress of Endocrine French Society, Deauville, France, September 29 to October 2, 2010.
Reprint requests: Jacques Young, M.D., Ph.D., Service d'Endocrinologie et des Maladies de la Reproduction, Hôpital Bicêtre, F-94275 Le Kremlin-Bicêtre, France (E-mail: jacques.young@bct.aphp.fr).

0015-0282/$36.00
doi:10.1016/j.fertnstert.2011.03.091

E_2 deficiency (5, 9). We also compared the effects of T enanthate, combined gonadotropin treatment, and dihydrotestosterone (DHT) administration on these patients' E_2 levels. Finally, we propose that this pathological model could be useful for deciphering the respective effects of T and E_2 on bone metabolism, independently of factors related to aging.

MATERIALS AND METHODS

Patients

This single-center study was approved by the institutional review boards of Bicêtre teaching hospital and Faculté de Médecine Paris Sud. All the patients and healthy volunteers gave their written informed consent. Men who used any anabolic medication were excluded from the study.

Untreated patients with CHH We included 91 previously untreated patients with CHH referred to the Reproductive Health and Endocrine Department of Bicêtre Hospital in Paris, France, between January 2001 and August 2010. These patients' isolated gonadotropin deficiency was characterized by [1] absent or incomplete puberty at age 17 years; [2] low serum T levels and low or normal serum gonadotropin levels; [3] normal basal and stimulated levels of cortisol (F), growth hormone, PRL, and TSH in response to insulin-induced hypoglycemia and thyrotropin-releasing hormone (TRH), and normal basal serum DHEAS and thyroid hormone levels; [4] normal serum insulin-like growth factor I (IGF-I), iron, and ferritin concentrations; and [5] normal magnetic resonance imaging (MRI) of the hypothalamic-pituitary region (1, 2). Forty-one of these patients were considered to have Kallmann syndrome, as olfactometry showed anosmia or hyposmia and/or MRI showed olfactory bulb aplasia or hypoplasia (10, 11). None of the patients in this group had previously received androgen or gonadotropin replacement therapy.

Healthy men Sixty-three men, 17 to 46 years of age, were evaluated in our department between March 2001 and May 2010 because they belonged to couples presenting with infertility of female origin. They were chosen for the evaluation of normal gonadotropin and gonadal steroid secretion on the basis of the following criteria (collected by J.Y.): no unusual history, sexual activity and physical examination, including testicular volume more than 15 mL (Prader orchidometer); normal serum concentrations of LH, FSH, and T; and normal semen analysis (>20 million sperm/mL, >50% motility, >2-mL volume).

Patients with Klinefelter syndrome For comparison with a classic testicular cause of hypogonadism, we also included 45 untreated patients with Klinefelter syndrome (34.5 ± 11.8 years; mean ± SD) who were referred to our department during the same period for pubertal delay, gynecomastia, or infertility. All of these patients had peripheral karyotyping, showing that all cells harbored a 47,XXY complement (homogenous Klinefelter syndrome).

The main characteristics of the three groups of subjects are summarized in supplemental Table 1 (available online).

Treated patients with CHH To evaluate the effect of T enanthate treatment on circulating E_2 levels, blood samples were drawn at random times during the same period from 101 patients with CHH managed in our department and who were receiving this drug (Androtardyl; Schering; 250 mg IM, every 3 weeks) as routine virilization therapy.

In addition, 88 subjects with CHH receiving a combination of hCG (Gonadotrophine-chorionique; Laboratoires Organon, Puteau, France; 1,500 IU IM, twice or three times a week) and recombinant or extractive human FSH (GONAL-f; Laboratoires Merck-Serono, Lyon, France or Menopur; Laboratoires Ferring Pharmaceuticals, Gentilly, France; both preparations 150 IU SC twice or three times a week), to induce spermatogenesis, were included to evaluate changes in circulating E_2 levels relative to untreated patients and those with CHH receiving T enanthate.

The effect of treatment on circulating total E_2 (TE2) levels could be prospectively evaluated in subgroups of subjects with CHH receiving T enanthate (n = 22; aged: 18–50 years; body mass index [BMI]: 25.6 ± 4.8) and hCG-FSH combined therapy (n = 18; aged: 18–54 years; BMI: 25.5 ± 4.4). Similarly, in 12 previously untreated patients with CHH (aged: 18–27 years; BMI: 24.7 ± 2.8) we were able to prospectively evaluate the effect on circulating TE2 levels of percutaneous daily administration for 1 month of the nonaromatizable androgen DHT (Andractim; Besins International, Paris, France; 250 mg/d in 5 g of gel) compared with T enanthate.

Assays

Serum sex hormone-binding globulin and T Serum sex hormone-binding globulin (SHBG) was measured with a solid-phase chemiluminescent immunometric assay (Immulite; Siemens Healthcare Diagnostic Products, Llanberis, United Kingdom) with a detection limit of 0.02 nmol/L. The intra-assay and interassay coefficients of variation (CV) were 3.2% and 4.6% for a SHBG concentration of 56.4 nmol/L (12).

Serum total T (TT) was measured with a direct radioimmunoassay on an Orion Diagnostica device (Spectria, Espoo, Finland) with a detection limit of 0.02 ng/mL (0.06 nmol/L). The intra-assay and interassay CVs were, respectively, 3.8% and 4.8% at 3.3 and 2.6 ng/mL (11.4 and 9.1 nmol/L) and the intra-assay and interassay CVs were 7.5% and 7.0% at, respectively, 0.46 and 0.35 ng/mL (1.6 and 1.2 nmol/L) for TT (13).

Serum concentrations of bioavailable and free T (BT and FT) were calculated with validated algorithms based on equations described by Vermeulen et al. (14), using measured TT and SHBG concentrations, an assumed constant for albumin (43 g/L), and affinity constants of SHBG and albumin for T. The FT fraction was determined with the FT calculation from Vermeulen et al. (14), owing to the poor reliability of commercial FT assays relative to the equilibrium dialysis method (14), as confirmed in the present study (data not shown).

Estradiol Serum total 17β-E_2 was measured with a sensitive direct RIA on an Orion Diagnostica device (Spectria). The detection limit was 2 pg/mL (7.3 pmol/L). The intra-assay and the interassay CVs were 2.8% and 5.8%, respectively, at 23.5 and 25.4 pg/mL (87 and 94 pmol/L) and the intra-assay and interassay CVs were 18.1% and 17.6% at 4.6 and 3.3 pg/mL (17 and 12 pmol/L for serum total 17β-E_2).

In 24 patients with CHH, serum TE2 values measured with RIA (range: 4–68 pg/mL, median 12 pg/mL) was compared with values obtained by the gas chromatography-tandem mass spectrometry method. An excellent correlation was found ($r = 0.969$; $P<.0001$) demonstrating the accuracy of the E_2 RIA method used in the present study.

The serum bioavailable 17β-E_2 (BE2) concentration was calculated with a validated algorithm, based on the equations of Södergard et al. (15) and using the measured TE2, TT, and SHBG concentrations, an assumed constant for the albumin concentration (43 g/L), and affinity constants of SHBG and albumin for 17β-E_2. This method has been shown to have high validity (16). We also measured BE2 by differential precipitation of globulin-bound E_2 with 50% ammonium sulfate, after equilibration of the serum sample with [^{3}H]-E_2 (17). We found that measured BE2 values correlated strongly with calculated BE2 values ($r = 0.97$, $P<.0001$) (Supplemental Fig. 1, available online), and therefore report only the calculated fraction of BE2, a parameter currently favored in the literature. The free fraction of 17β-E_2 (FE2) was calculated with the algorithm of Södergard et al. (15), as previously described.

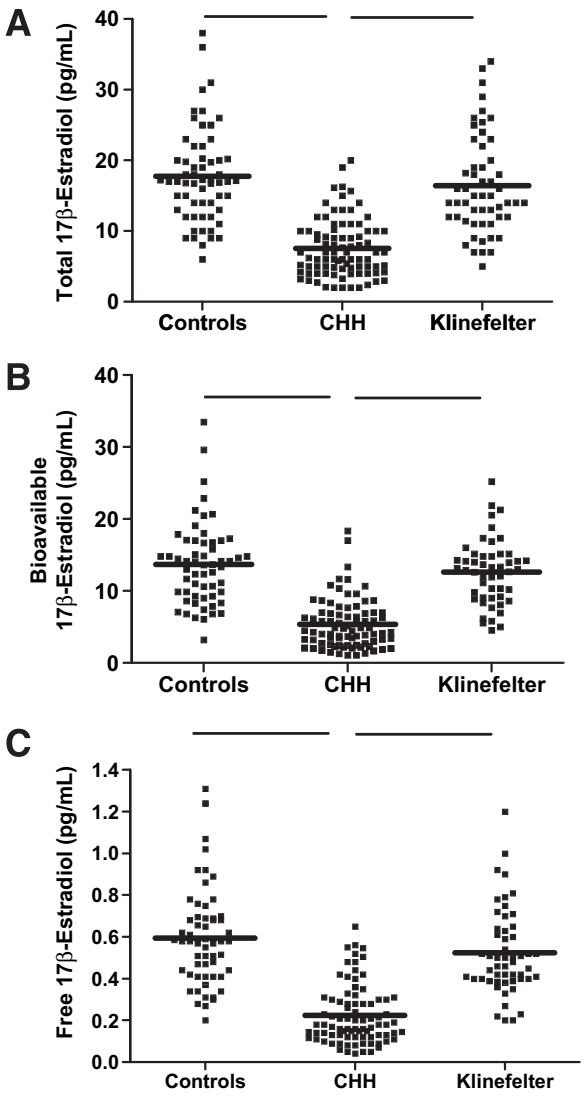
Gonadotropins The FSH and LH levels were measured with an ultrasensitive RIA as described (10, 11, 18) on an Immunotech device (Beckman Coulter, Praha, Czech Republic). The detection limits were 0.05 IU/L for both FSH and LH. The intra-assay and interassay CVs were no more than 6.3% for FSH (10, 11, 18) and no more than 6.7% for LH (10, 11, 18).

Statistical Analysis

All results are reported as individual values in the figures and as mean ± SD in the tables and text. We used one-way repeated measures analysis of variance (ANOVA) to assess differences across the groups, followed by appropriate post hoc comparisons. Hormonal parameters were compared by using a parametric t test or the Mann-Whitney, Wilcoxon, or Kolmogorov-Smirnov nonparametric tests. P values less than .05 were considered to denote significant differences.

FIGURE 1

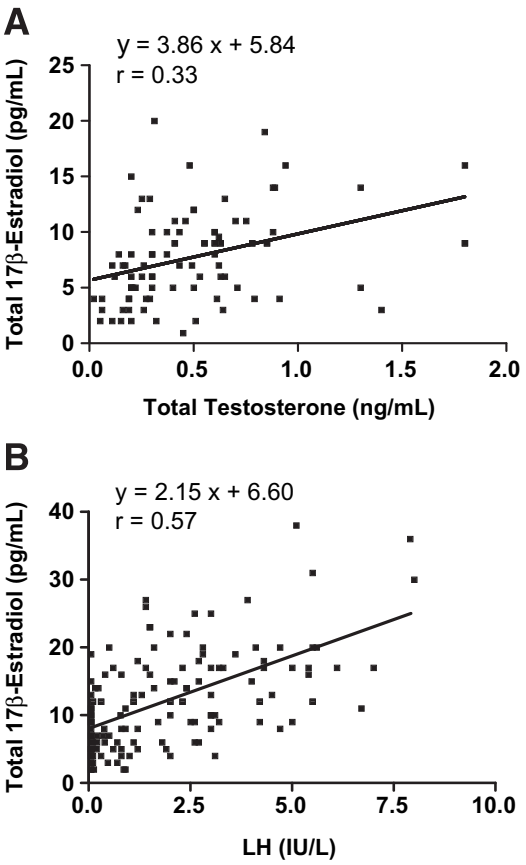
Total (**A**), bioavailable (**B**), and free (**C**) E_2 levels in healthy young men (controls) and untreated young patients with CHH and Klinefelter syndrome. $P<.001$ for all groups. Conversion to SI units: To convert total, bioavailable and free E_2 concentration from pg/mL to picomoles per liter, multiply by 3.671.



Trabado. E_2 deficiency in male CHH. Fertil Steril 2011.

FIGURE 2

(**A**) Correlation between circulating total testosterone and circulating total E_2 levels in untreated patients with CHH; $P=.003$. (**B**) Correlation between serum LH and circulating total E_2 levels in the combined population of controls and untreated patients with CHH; $P<.001$.



Trabado. E_2 deficiency in male CHH. Fertil Steril 2011.

RESULTS

Serum SHBG and Circulating T in Controls and Patients With CHH and Klinefelter Syndrome

Mean SHBG levels in each group are shown in Supplemental Table 1. Interestingly, despite their higher BMI (19), the patients with CHH (36.2 ± 21.4 nmol/L) had significantly higher circulating SHBG levels than controls (28.6 ± 10.4 nmol/L), possibly because of weaker SHBG repression owing to low T levels (19).

Mean (±SD) total, BT, and FT values in the controls, patients with CHH, and those with Klinefelter syndrome are shown in Supplemental Table 1. As expected (1, 2), TT levels were far lower in untreated patients with CHH than in the controls. Circulating T levels were also far lower than control values when BT or FT was used as an indicator of androgen deficiency. Compared with patients with CHH, patients with Klinefelter syndrome had far higher TT, BT, and FT levels. They also had a wider range of values, indicating a broader spectrum of T deficiency.

Circulating E_2

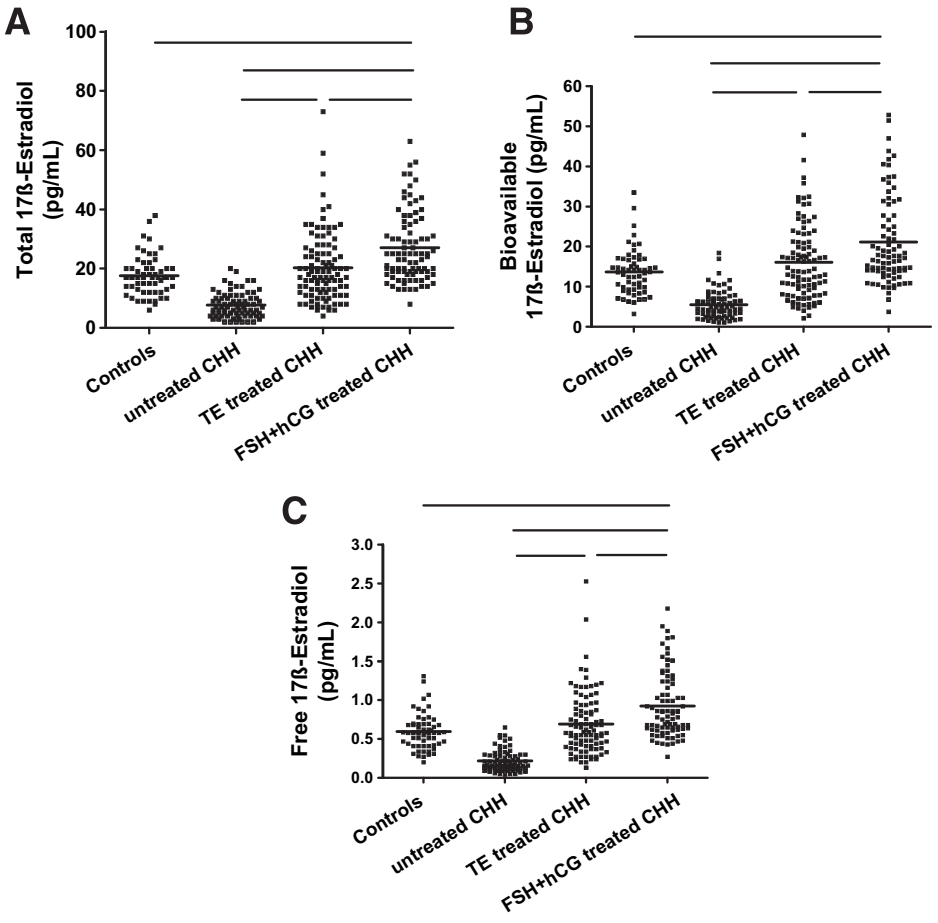
Individual values of TE2, BE2, and FE2 in the controls, patients with CHH, and those with Klinefelter syndrome are shown in Figure 1. The TE2, BE2, and FE2 were significantly lower in the patients with CHH than in the healthy controls (Fig. 1) and the patients with Klinefelter syndrome. All E_2 fraction levels were normal in the patients with Klinefelter syndrome.

Correlations Among TT, Serum LH, and Total E_2

As shown in Figure 2A, there was a positive and very significant correlation between serum TT and serum TE2 in the patients with CHH. In addition, individual serum TE2 values correlated strongly with serum LH values in the combined population of patients with CHH and controls (Fig. 2B).

FIGURE 3

Total E_2 (**A**), bioavailable E_2 (**B**), and free E_2 (**C**) in patients with CHH receiving either testosterone enanthate (TE) or FSH–hCG combination therapy, by comparison with untreated patients with CHH and healthy men (controls); $P<.001$ for all groups. To convert total, bioavailable, and free E_2 concentration from picograms per milliliter to picomoles per liter, multiply by 3.671.



Trabado. E_2 deficiency in male CHH. Fertil Steril 2011.

Circulating T and E_2 in Patients With CHH Treated With T Enanthate, the hCG-FSH Combination, or Percutaneous DHT

As expected, mean (±SD) TT (7.35 ± 4.71 ng/mL and 6.57 ± 2.43 ng/mL), BT (4.60 ± 3.26 ng/mL and 3.87 ± 1.81 ng/mL), and FT (0.16 ± 0.12 ng/mL and 0.14 ± 0.07 ng/mL) levels were significantly higher in patients with CHH receiving T enanthate or combined gonadotropin therapy than in untreated patients with CHH (respectively, 0.46 ± 0.35 ng/mL, 0.23 ± 0.21 ng/mL, and 0.01 ± 0.01 ng/mL; $P<.05$). No significant difference in TT levels was observed between patients with CHH treated with T enanthate and those receiving combined gonadotropin therapy, whereas BT and FT levels were slightly but significantly higher in men receiving T enanthate ($P<.05$).

Individual values of circulating E_2 fractions in patients with CHH receiving T enanthate or combined gonadotropin therapy are shown in Figure 3. Compared with untreated patients with CHH, mean (±SD) TE2, BE2, and FE2 levels were far higher in patients with CHH receiving either T enanthate (TE2: 20.2 ± 11.8 pg/mL; BE2: 16.1 ± 9.3 pg/mL; and FE2: 0.69 ± 0.39 pg/mL) or combined gonadotropin therapy (TE2: 27.0 ± 11.9 pg/mL; BE2: 21.1 ± 11.0 pg/mL; and FE2: 0.92 ± 0.42 pg/mL).

Levels of all circulating E_2 fractions in patients with CHH treated with T enanthate were similar to control values. In contrast, mean TE2, BE2, and FE2 levels were slightly but significantly higher in patients with CHH receiving combined gonadotropin therapy than in the control group and in T enanthate-treated men (Fig. 3).

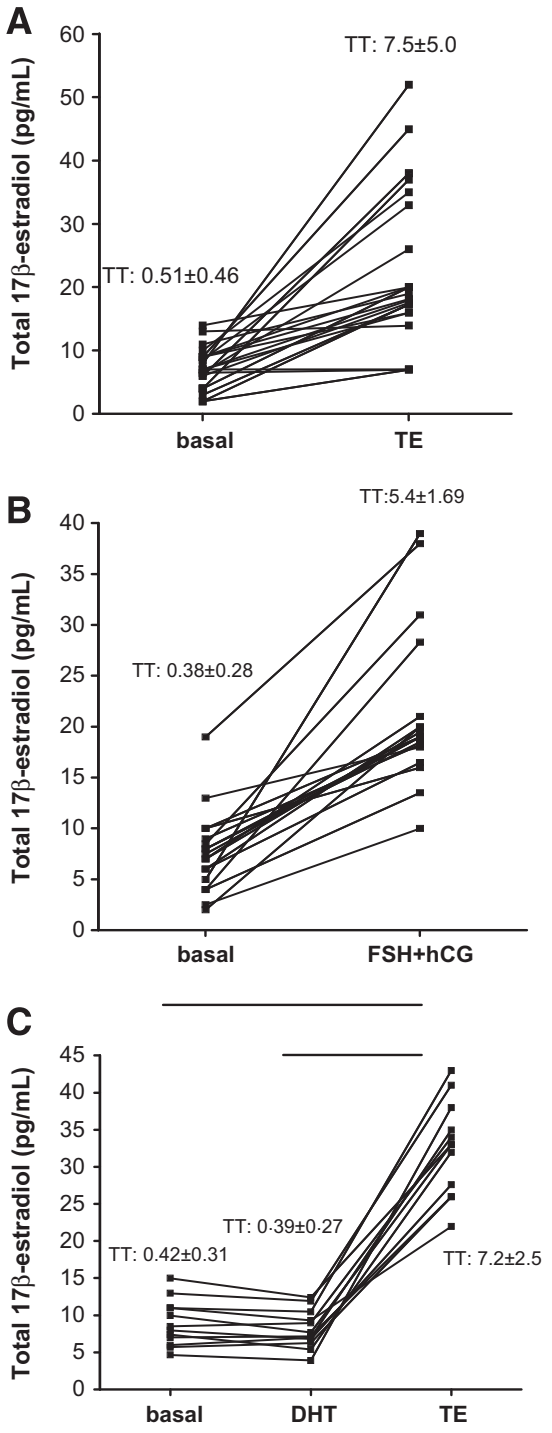
We had the opportunity to prospectively follow individual changes in TE2 levels in patients with CHH receiving T enanthate or combined gonadotropin treatment. As shown in Figure 4, TE2 increased markedly during both T enanthate (Fig. 4A) and combined gonadotropin therapy (Fig. 4B). On the contrary, as shown in Figure 4C, TE2 levels remained low during DHT therapy but increased markedly during T enanthate therapy in the same patients with CHH.

TE2:TT Ratio in Controls and T Enanthate or FSH + hCG Treated Patients With CHH

The TE2:TT ratio was not significantly different between the controls and T enanthate-treated patients with CHH (Supplemental Fig. 2, available online). In contrast, the TE2:TT ratio was slightly

FIGURE 4

Effect of testosterone enanthate (TE) (**A**) and FSH-hCG combined therapy (**B**) on circulating total E_2 levels in patients with CHH; $P<.0001$. (**C**) Effect of percutaneous dihydrotestosterone (DHT) administration followed by TE therapy on circulating total E_2 levels in patients with CHH; $P<.001$. Mean (±SD, nanograms per milliliter) serum total T (TT) before and under therapy is indicated (TT normal range in healthy men: 2.8–8.9 ng/mL). To convert total E_2 concentration from picograms per milliliter to picomoles per liter, multiply by 3.671.



Trabado. E_2 deficiency in male CHH. Fertil Steril 2011.

but very significantly increased in men receiving combined gonadotropin treatment when compared with T enanthate-treated patients and controls.

DISCUSSION

The main aim of this study was to test, in a large series, the hypothetical (5, 9) existence of marked E_2 deficiency in young men with untreated CHH and to evaluate its severity by measuring different fractions of circulating E_2. We clearly observed a severe E_2 deficiency in untreated patients with CHH, whatever the parameter used to evaluate body E_2 exposure.

As shown by the positive correlation between TE2 and TT, the E_2 deficiency in untreated patients with CHH was clearly linked to their T deficiency, in keeping with the substrate-product relationship between these two sex steroids. We also found that the decline in TE2 correlated with the decline in circulating LH, which is logical given the key role of this gonadotropin in the positive control of E_2 secretion by Leydig cells (20, 21), and suggests that the E_2 deficiency is linked to the circulating LH deficiency.

Interestingly, however, TE2 concentrations were not always very low in patients with very low LH concentrations (Fig. 2B). The persistence of noteworthy E_2 levels in patients with CHH with very low LH levels could be due to DHEAS conversion into E_2 (22, 23), as all the patients with CHH studied in the present study had normal concentrations of this adrenal precursor (data not shown).

We also demonstrate that the E_2 deficiency in men with CHH is far more severe than in men with Klinefelter syndrome. In these latter patients the E_2 deficiency correlates with the T deficiency, which is neither as consistent nor as severe as in CHH (24–26). We are currently comparing mineral bone density in young men affected by these two forms of male hypogonadism (Maione et al., work in progress).

In patients with CHH treated with an aromatizable androgen, we found that the three fractions of circulating E_2 increased significantly, approaching those in healthy men. This result is in keeping with the T-induced correction of osteopenia observed in some studies of few patients with CHH (4, 5). Contrary to treatment with T, an aromatizable androgen, we found that percutaneous DHT enanthate therapy did not correct the E_2 deficiency in patients with CHH. Treatment with this nonaromatizable androgen would therefore be inappropriate for men with CHH, as it could potentially prolong the E_2 deficiency and thereby fail to improve, or even aggravate, the osteopenia or osteoporosis of these patients. The situation is different in patients with Klinefelter syndrome in whom E_2 deficiency is often less severe and gynecomastia prevalent (24–26). In such patients it may be better to attempt to correct the abnormal breast development with percutaneous DHT, as this nonaromatizable androgen would not compromise mineral bone density (27, 28).

It is interesting to note that gonadotropin therapy increased E_2 levels significantly more than T enanthate therapy. This could be related to preferential stimulation of the aromatase of Leydig cells chronically stimulated by hCG (29) and might explain the gynecomastia observed during treatment with hCG, both alone and combined with FSH (30). Additional work is needed to determine whether combined gonadotropin therapy is superior to T ester administration with respect to acquisition of mineral bone density in adolescents and young men with CHH.

Most studies of the skeletal effects of androgens and estrogens have focused on elderly subjects (7, 8), and it is possible that

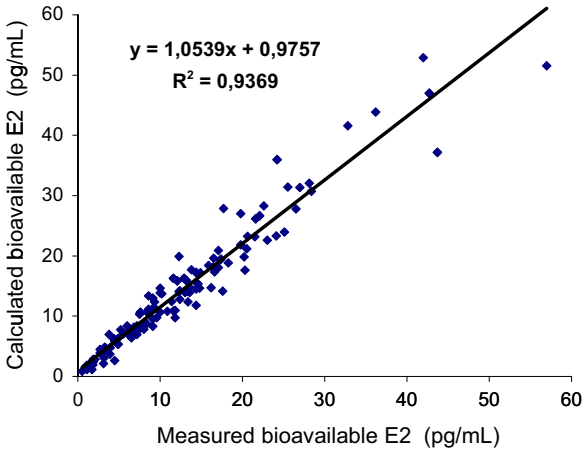
nonspecific factors linked to other hormone deficiencies or to age-related morbidities masked the specific effects of E_2 on bone metabolism. The model of CHH would thus be useful for studying the specific effects of androgens and estrogens on skeletal development, especially in the second and third decades when the bulk of mineral bone density is acquired (31, 32).

REFERENCES

1. Brioude F, Bouligand J, Trabado S, Francou B, Salenave S, Kamenicky P, et al. Non-syndromic congenital hypogonadotropic hypogonadism: clinical presentation and genotype-phenotype relationships. Eur J Endocrinol 2010;162:835–41.
2. Seminara SB, Hayes FJ, Crowley WF. Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. Endocrinol Rev 1998;19: 521–9.
3. Finkelstein JS, Klibanski A, Neer RM, Greenspan SL, Rosenthal DI, Crowley WF. Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. Ann Intern Med 1987;106:354–61.
4. Finkelstein JS, Klibanski A, Neer RM, Doppelt SH, Rosenthal DI, Segre GV, et al. Increases in bone density during treatment of men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 1989;69:776–83.
5. Behre HM, Kliesch S, Leifke E, Link TM, Nieschlag E. Long-term effect of testosterone therapy on bone mineral density in hypogonadal men. J Clin Endocrinol Metab 1997;82:2386–90.
6. Gennari L, Nuti R, Bilezikian JP. Aromatase activity and bone homeostasis in men. J Clin Endocrinol Metab 2004;89:5898–907.
7. Khosla S, Amin S, Orwoll E. Osteoporosis in men. Endocrinol Rev 2008;29:441–64.
8. Khosla S. Update in male osteoporosis. J Clin Endocrinol Metab 2010;95:3–10.
9. Okuyama A, Nakamura M, Namiki M, Aono T, Matsumoto K, Utsunomiya M, et al. Testicular responsiveness to long-term administration of hCG and hMG in patients with hypogonadotrophic hypogonadism. Horm Res 1986;23:21–30.
10. Sarfati J, Guiochon-Mantel A, Rondard P, Arnulf I, Garcia-Piñero A, Wolczynski S, et al. A comparative phenotypic study of Kallmann syndrome patients carrying monoallelic and biallelic mutations in the prokineticin 2 or prokineticin receptor 2 genes. J Clin Endocrinol Metab 2010;95:659–69.
11. Salenave S, Chanson P, Bry H, Pugeat M, Cabrol S, Carel JC, et al. Kallmann's syndrome: a comparison of the reproductive phenotypes in men carrying KAL1 and FGFR1/KAL2 mutations. J Clin Endocrinol Metab 2008;93:758–63.
12. Raven G, De Jong FH, Kaufman JM, De Ronde W. In men, peripheral estradiol levels directly reflect the action of estrogens at the hypothalamo-pituitary level to inhibit gonadotropin secretion. J Clin Endocrinol Metab 2006;91:3324–8.
13. Lapauw B, Taes Y, Goemaere S, Toye K, Zmierczak HG, Kaufman JM. Anthropometric and skeletal phenotype in men with idiopathic osteoporosis and their sons is consistent with deficient estrogen action during maturation. J Clin Endocrinol Metab 2009;94:4300–8.
14. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.
15. Södergard R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982;16:801–10.
16. Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev 2002;11:1065–71.
17. Loric S, Guechot J, Duron F, Aubert P, Giboudeau J. Determination of testosterone in serum not bound by sex-hormone-binding globulin: diagnostic value in hirsute women. Clin Chem 1989;34:1826–9.
18. Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, et al. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med 2009;360:2742–8.
19. Pugeat M, Crave JC, Elmidani M, Nicolas MH, Garoscio-Cholet M, Lejeune H, et al. Pathophysiology of sex hormone binding globulin (SHBG): relation to insulin. J Steroid Biochem Mol Biol 1991;40:841–9.
20. Winters SJ, Troen P. Testosterone and estradiol are co-secreted episodically by the human testis. J Clin Invest 1986;78:870–3.
21. Young J, Couzinet B, Chanson P, Brailly S, Loumaye E, Schaison G. Effects of human recombinant luteinizing hormone and follicle-stimulating hormone in patients with acquired hypogonadotropic hypogonadism: study of Sertoli and Leydig cell secretions and interactions. J Clin Endocrinol Metab 2000;85:3239–44.
22. Arlt W, Haas J, Callies F, Reincke M, Hübler D, Oettel M, et al. Biotransformation of oral dehydroepiandrosterone in elderly men: significant increase in circulating estrogens. J Clin Endocrinol Metab 1999;84:2170–6.
23. Young J, Couzinet B, Nahoul K, Brailly S, Chanson P, Baulieu EE, et al. Panhypopituitarism as a model to study the metabolism of dehydroepiandrosterone (DHEA) in humans. J Clin Endocrinol Metab 1997;82:2578–85.
24. Smyth CM, Bremner WJ. Klinefelter syndrome. Arch Intern Med 1998;158:1309–14.
25. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. Lancet 2004;364:273–83.
26. El Amm M, Brailly S, Bauduceau B, Young J. Klinefelter syndrome. In: Chanson P, Young J, editors. Endocrinology treatise. Paris: Flammarion; 2007. p. 622–7.
27. Kuhn JM, Rieu M, Laudat MH, Forest MG, Pugeat M, Bricaire H, et al. Effects of 10 days administration of percutaneous dihydrotestosterone on the pituitary-testicular axis in normal men. J Clin Endocrinol Metab 1984;58:231–5.
28. Kuhn JM, Roca R, Laudat MH, Rieu M, Luton JP, Bricaire H. Studies on the treatment of idiopathic gynaecomastia with percutaneous dihydrotestosterone. Clin Endocrinol (Oxf) 1983;19:513–20.
29. Rieu M, Reznik Y, Vannetzel JM, Mahoudeau J, Berrod JL, Kuhn JM. Testicular steroidogenesis in adult men with human chorionic gonadotropin-producing tumors. J Clin Endocrinol Metab 1995;80:2404–9.
30. Schopohl J, Mehltretter G, von Zumbusch R, Eversmann T, von Werder K. Comparison of gonadotropin-releasing hormone and gonadotropin therapy in male patients with idiopathic hypothalamic hypogonadism. Fertil Steril 1991;56: 1143–50.
31. Handelsman DJ, Liu PY. Klinefelter's syndrome—a microcosm of male reproductive health. J Clin Endocrinol Metab 2006;91:1220–2.
32. Aminorroaya A, Kelleher S, Conway AJ, Ly LP, Handelsman DJ. Adequacy of androgen replacement influences bone density response to testosterone in androgen-deficient men. Eur J Endocrinol 2005;152:881–6.

SUPPLEMENTAL FIGURE 1

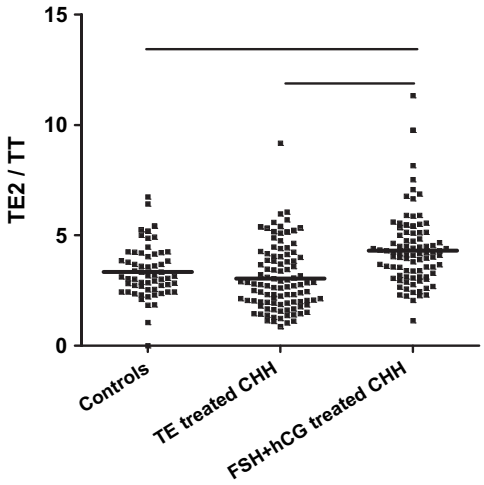
In patients with CHH, correlation between bioavailable E_2 (BE2) measured by differential precipitation of globulin-bound E_2 with 50% ammonium sulfate, after equilibration of the serum sample with [^{3}H]-E_2 and calculated bioavailable E_2 based on the equations of Södergard et al. (15).



Trabado. E_2 deficiency in male CHH. Fertil Steril 2011.

SUPPLEMENTAL FIGURE 2

Serum total E_2 to serum total T (TE2/TT) ratio in healthy men (controls) and in T enanthate-treated (TE) or FSH+hCG-treated patients with CHH; $P < .001$.



Trabado. E_2 deficiency in male CHH. Fertil Steril 2011.

SUPPLEMENTAL TABLE 1

Main characteristics of healthy men, untreated patients with CHH and Klinefelter syndrome.

Variable	Controls	CHH	Klinefelter
n	63	91	45
Age (y)	34.0 ± 11.4 (17–46)	28.8 ± 10.1 (17–45)	34.5 ± 11.8 (17–51)
BMI (kg/m^2)	22.8 ± 1.9 (18.6–27.1)	25.4 ± 5.4[a] (17.5–40.1)	25.0 ± 5.0[c] (17.0–33.2)
SHBG (nmol/L)	28 ± 10 (13–56)	36 ± 22[a] (3–107)	27.5 ± 15.1 (10–78)
FSH (IU/L)	3.5 ± 1.8 (1.3–8.6)	0.78 ± 0.8[b] (0.05–3.8)	31.0 ± 15.3[d] (12–78)
LH (IU/L)	3.5 ± 1.7 (1.6–8.0)	0.59 ± 0.7[b] (0.05–3.1)	17.4 ± 7.9[d] (9.0–39.0)
Total T (ng/mL)	5.2 ± 1.2 (3.4–8.9)	0.46 ± 0.4[b] (0.02–1.8)	1.8 ± 1.2[d,e] (0.2–4.8)
Bioavailable T (ng/mL)	3.0 ± 0.8 (1.8–5.6)	0.23 ± 0.2[b] (0.01–0.9)	1.0 ± 0.5[d,e] (0.1–2.0)
Free T (ng/mL)	0.11 ± 0.03 (0.07–0.20)	0.009 ± 0.01[b] (ND–0.03)	0.03 ± 0.02[d,e] (ND–0.07)
Total E_2 (pg/mL)	17.6 ± 6.6 (6–38)	7.65 ± 4.2[b] (2–19)	16.0 ± 7.2[e] (5–34)
Bioavailable E_2 (pg/mL)	13.7 ± 5.7 (3.2–33.5)	5.3 ± 3.6[b] (1.1–17.4)	12.4 ± 5.7[e] (4.6–25.3)
Free E_2 (pg/mL)	0.60 ± 0.23 (0.2–1.3)	0.21 ± 0.13[b] (0.05–0.65)	0.53 ± 0.22[e] (0.2–1.0)

Note: Data are expressed as mean ± SD (range: min-max). To convert total, bioavailable, and free T from nanograms per milliliter to nanomoles per liter, multiply by 3.467; to convert total, bioavailable, and free E_2 concentration from picograms per milliliter to picomoles per liter, multiply by 3.671. BMI = body mass index; SHBG = sex hormone–binding globulin; ND = not detectable.

[a] $P<.01$ CHH versus controls.

[b] $P<.0001$ CHH versus controls.

[c] $P<.01$ Klinefelter versus controls.

[d] $P<.0001$ Klinefelter versus controls.

[e] $P<.001$ Klinefelter versus CHH.

Trabado. E_2 deficiency in male CHH. Fertil Steril 2011.