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CLINICAL CASE SEMINAR

Dysmetabolic Syndrome in a Man with a Novel Mutation of the Aromatase Gene: Effects of Testosterone, Alendronate, and Estradiol Treatment

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We present the fourth case of an adult man (29 yr old) affected by aromatase deficiency resulting from a novel homozygous inactivating mutation of the CYP19 ($P450_{arom}$) gene. At first observation, continuing linear growth, eunuchoid body proportions, diffuse bone pain, and bilateral cryptorchidism were observed. The patient presented also a complex dysmetabolic syndrome characterized by insulin resistance, diabetes mellitus type 2, acanthosis nigricans, liver steatohepatitis, and signs of precocious atherogenesis. The analysis of the effects induced by the successive treatment with high doses of testosterone, alendronate, and estradiol allows further insight into the roles of androgens and estrogens on several metabolic functions. High doses of testosterone treatment resulted in a severe imbalance in the estradiol to testosterone ratio together with the occurrence of insulin resistance and diabetes mellitus type 2. Estrogen treatment resulted in an improvement of acanthosis nigricans, insulin resistance, and liver steatohepatitis, coupled with a better glycemic control and the disappearance of two carotid plaques. Furthermore, the study confirms previous data concerning the key role of estrogens on male bone maturation, at least in part, and regulation of gonadotropin secretion. The biopsy of the testis showed a pattern of total germ cell depletion that might be due to the concomitant presence of bilateral cryptorchidism. Thus, a possible role of estrogen in male reproductive function is suggested but without revealing a direct cause-effect relationship.

Data from this case provide new insights into the role of estrogens in glucose, lipid, and liver metabolism in men. This new case of aromatase deficiency confirms previous data on bone maturation and mineralization, and it reveals a high risk for the precocious development of cardiovascular disease in young aromatase-deficient men. (*J Clin Endocrinol Metab* 89: 61–70, 2004)

FROM THE STUDY of estrogen-deficient male mice (1, 2) and men affected by estrogen deficiency due to mutations either of the estrogen receptor-α gene (3) or the aromatase gene (4–8), it was established that estrogen is necessary to achieve epiphyseal closure and peak bone mass in the male (9). Additionally, the role of estrogen on both bone and gonadotropin feedback regulation in men has been confirmed during adulthood and aging in men (10–15). It follows that the role of androgens is mediated, at least in part, by conversion to estrogens, probably in cells of the bone itself (10–12) and/or at the hypothalamic-pituitary level (13–15).

Clinical features of men affected by congenital estrogen deficiency are tall stature due to incomplete epiphyseal closure, osteoporosis, and eunuchoid proportions of the skeleton (9). Transdermal estradiol (5) or conjugated estrogen (6) supplementation leads to bone maturation after complete epiphyseal closure and to an increase in bone mineral density (BMD). Less is known about the effects of estrogen treatment on metabolic parameters. In the patient of Bilezikian *et al.* (6), serum insulin concentrations were elevated, and these fell to within the normal range upon treatment with conjugated estrogens. Elevated insulin resistance was recently documented in another patient (8), but the issue concerning a possible direct estrogen action on insulin and glucose secretion remains unclear.

Here we report a new case of a man with a novel homozygous inactivating mutation of the *CYP19* ($P450_{arom}$) gene and with a marked metabolic phenotype. The effects on metabolic parameters, bone maturation, BMD, and endocrine parameters of three different long-term treatments are evaluated and compared. This patient, in fact, was treated for an extended period of time with androgens, spaced out by alendronate, before the diagnosis of aromatase deficiency was made. Finally, after the diagnosis he was treated with estrogens. The novel aspects of this case include the role of estrogen deficiency and estrogen treatment in glucose, lipid, and liver metabolism.

Abbreviations: ArKO, Aromatase knockout; BMD, bone mineral density; BMI, body mass index; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GT, γ-glutamyl-transferase; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NASH, nonalcoholic steatohepatitis.

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Case Report

The patient, a 29-yr-old, 46-XY Caucasian male with a normal karyotype at the time of the diagnosis of aromatase deficiency, came to our attention in 1996, at the age of 25 yr, because of persistent linear growth and diffuse bone pain, particularly at the appendicular skeleton. The patient had a history of bilateral cryptorchidism, which was treated surgically at the age of 6 yr without success.

The height of the patient was 172 cm at the age of 21 yr and linear growth continued during adulthood. Upon physical examination, the patient was 177 cm tall and his weight was 79.6 kg at the time of first observation. Neither target height nor the patient's pedigree could be established because the patient was an orphan and reared by unrelated persons. The patient apparently had normal growth during infancy and pubertal development.

He had bilateral genu valgum and a eunuchoid skeleton (the ratio of the upper to the lower segment of the skeleton was 0.84; average for men is 0.92), which became rapidly more evident after the age of 23 yr (Fig. 1). Virilization was normal in terms of pubic hair. Gynecomastia was absent but a gynoid body fat distribution was evident. The penis was of normal size without hypospadias. Right and left testes with volumes of 10 and 11 ml, respectively, were in the inguinal canal and presented with a very soft consistency.

As assessed by a detailed sexological interview (BEM Sex Role Inventory and a self-filled daily diary), gender identity was normal, sexual orientation was heterosexual, and libido and sexual function were unaffected, even though the patient denied sexual intercourse according to his religious belief (he is a Roman Catholic priest).

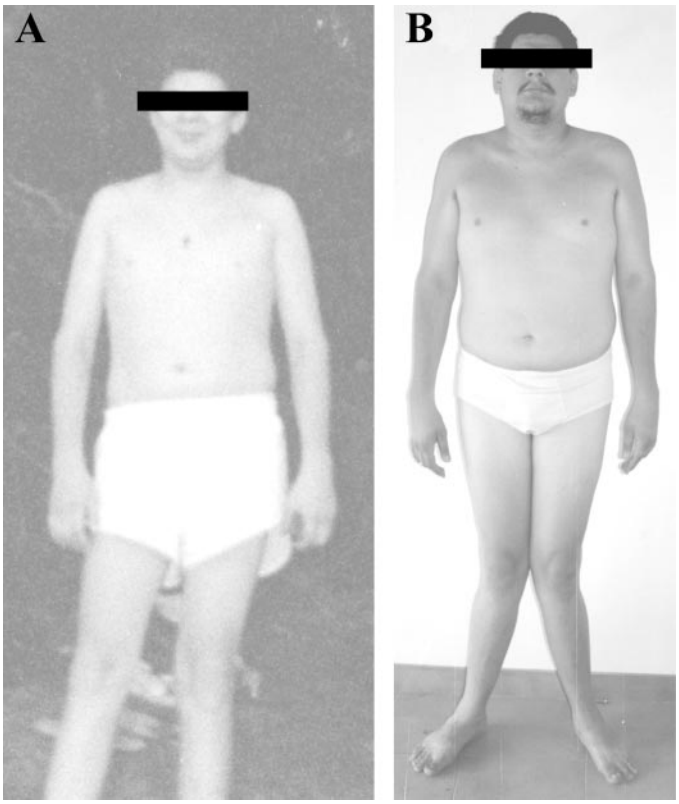

FIG. 1. A, Patient at the beginning of the clinical history, at the age of 23 yr, before the first visit. B, Patient at the time of the diagnosis of aromatase deficiency before estrogen treatment, phase 5 (29 yr old). Eunuchoid body proportions and genu valgum are present at 23 yr and worsened notwithstanding 8 and 13 months of high dose of testosterone treatment spaced out from 10 months of withdrawal.

The clinical study was based on data obtained before and after the diagnosis of aromatase deficiency. Results obtained before the diagnosis are from observational retrospective data. After the diagnosis, a prospective protocol, based on three subsequent phases according to the treatments performed, was designed to study the effects of estrogen treatment on several clinical parameters. After giving informed consent, the patient underwent the following treatments:

Before the diagnosis of aromatase deficiency (retrospective data): Phase 1: Before treatments. Phase 2: Testosterone enanthate (Testoviron depot), 250 mg im for 14 months, every 21 d for 6 months and every 15 d for 8 months. Phase 3: Alendronate, 10 mg daily for 10 months. Phase 4: Transdermal testosterone, 6 mg daily for an additional 13 months. [It should be noted that both testosterone treatments (phases 2 and 4) were performed using supraphysiological doses.]

After the diagnosis of aromatase deficiency (prospective study). Phase 5: No treatment for 9 months. Phase 6: Transdermal estradiol (Estraderm TTS), 25 μg twice weekly for 6 months. Phase 7: Transdermal estradiol, 50 μg twice weekly for 6 months.

During each phase of treatment, hormonal and biochemical studies were performed together with a radiological evaluation of the hand for bone age and BMD measurement. During the whole study, the patient received vitamin D (400 IU daily) plus calcium (1 g daily) supplementation.

Materials and Methods

DNA sequencing

Each coding exon (exons II-to-X) of the *CYP19* gene, along with untranslated exon I.4 and its 5′ flanking region, was amplified by PCR from genomic DNA isolated from the patient's blood. The primer sequences used have been described elsewhere (16). Purified PCR fragments were either sequenced directly or subcloned into the pGEM-T Easy Vector (Promega, Madison, WI) before sequencing (ABI Prism 377 DNA sequencer).

Expression of aromatase mutant cDNA

Both wild-type and mutant human *CYP19* cDNAs were prepared by PCR amplification from the plasmid pCMV.arom (17) introducing the desired mutation by primer-directed mutagenesis. These cDNAs were subcloned into the expression vector pcDNA3.1(+) (Invitrogen, Carlsbad, CA) and transiently transfected into COS-1 cells using Fugene reagent (Roche Diagnostics, Basel, Switzerland). Aromatase activity was determined 24 h later by the production of ^3H_2O from the substrate [1β-^{3}H]androstenedione (18).

Evaluation of the integrity of the splicing junction

To evaluate the integrity of the splicing junction, an exon-trapping system (Life Technologies, Grand Island, NY) was used. This system allows isolation of spliced cDNA products from cloned genomic DNA. Exon V and its flanking regions from both the patient and a control subject were subcloned into the exon-trapping vector pSPL3. These plasmids were transfected into COS-7 cells, and after 18 h of expression total RNA was isolated. After first-strand cDNA synthesis, the spliced products were amplified by PCR. The size of the PCR products obtained indicated whether splicing had occurred between expected splice sites within the subcloned genomic DNA and pSPL3 vector.

To further confirm that correct splicing had occurred, these fragments were subcloned into the pAMP10 cloning vector for DNA sequencing. Sequencing was performed as described above.

Hormonal and biochemical studies

During each phase of treatment, a blood sample was taken from the subject starting at 0800 h after an overnight fast, and serum samples were stored at −80 C until assayed. The following parameters were measured: Serum LH and FSH were measured by a fluoroimmunoassay (Autodelfia hLH kit, Wallac Oy, Turku, Finland) with a sensitivity of 0.05 IU/liter. The interassay and the intraassay coefficients of variation were 3.6 and 2.8%, respectively, for LH and 4.1 and 2.6%, respectively, for FSH. Serum total testosterone was measured by commercial RIA (Diagnostics Product Corp., Los Angeles, CA). The interassay and the intraassay coefficients of variation for testosterone were 11 and 5%, respectively. Serum estradiol was detected employing a commercially available double antibody RIA (Third-Generation DSL-39100, Diagnostic Systems Laboratories, Inc., Webster, TX). Sensitivity was 0.6 pg/ml (2.2 pmol/liter) with the lowest standard at 1.5 pg/ml, linearity to 150 pg/ml, and an ED_{50} of 20 pg/ml. The cross-reactivity with estrone and with less potent estrogens was less than 7 and 0.45%, respectively. The interassay and the intraassay coefficients of variation for estradiol were 4.1–9.9 and 3.4–3.9%, respectively.

Prolactin, insulin, urinary deoxypyridinoline, osteocalcin, PTH and bone alkaline phosphatase, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, glycosylated hemoglobin (HbA1c), fructosamine, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), γ-glutamyl-transferase (γ-GT), alkaline phosphatase, calcium, and phosphate were assayed by means of commercially available kits. An oral glucose tolerance test was not performed because diabetes mellitus occurred in phase 4.

Bone densitometry

BMD was assessed at the lumbar spine (L2–L4) and the femoral neck in every phase of the study protocol using dual-electron x-ray absorptiometry (Lunar DPX-L, Lunar Corp., Madison, WI).

X-ray film

X-ray films of wrist and hand were performed during each phase of the treatment.

Metabolic outcomes

An echo-Doppler study of the carotid arteries was performed during phases 5, 6, and 7.

Histological study

A biopsy of the testis, skin, and liver was performed.

Ethics

The patient gave his informed consent to the treatment and the publication of the data and pictures.

Results

Aromatase gene mutation

Sequencing of the aromatase gene revealed a novel homozygous point mutation at the last nucleotide of exon V (G→A). This single base mutation would be expected to change the corresponding amino acid from an acidic residue to a basic residue, Glu210Lys. *In vitro* activity of the mutant aromatase was measured and compared with a normal aromatase dose-response curve. Results showed that this amino acid substitution does not alter the aromatase activity in the radiometric assay (Fig. 2A).

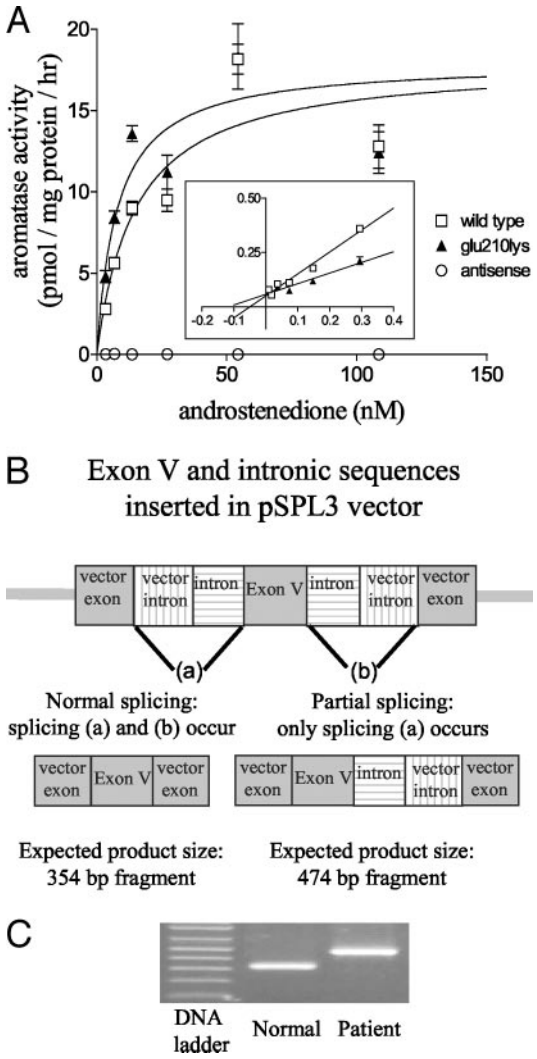

FIG. 2. Aromatase gene mutation analysis. A, Activity of Glu210Lys and wild-type aromatase proteins. Expression constructs encoding either *CYP19* genotype were transiently transfected into nonsteroidogenic COS-1 cells. Cells were incubated with various concentrations of [^{3}H]androstenedione for 2 h and aromatase activity detected as the release of ^3H_2O. *Inset*, Lineweaver-Burke transformation indicated that the calculated kinetic parameters for wild-type and mutant proteins were not significantly different: (Vmax = 14.7 *vs.* 14.9 pmol/mg protein·h; Km = 13.7 *vs.* 7.8 nM, respectively). B, Schematic splicing events in the exon-trapping procedure. The patient's and a normal exon V fragment with its flanking region were subcloned into the exon-trapping vector, pSPL3. They were transfected into COS-7 and first-strand cDNA was synthesized from isolated total RNA. C, A predicted 354-bp fragment, spliced in both 5′and 3′ ends, is shown as a product of the normal aromatase exon V fragment. The exon V fragment isolated from the patient was able to generate only one 474-bp transcript, which is spliced at the 5′ end but not at the 3′ end.

This mutation (..ACG/gtactg.. to ..ACA/gtactg) would also lead to an aberrant splicing of the mRNA. Consensus of the splicing site is highly conserved only immediately within the intron at the presumed junctions. Consequently, an exon-trapping system was used to verify the hypothesis that the donor-splicing site in exon V was destroyed. Results confirmed that the spliceosome was unable to recognize a splicing site (Fig. 2, B and C). Consequently, the mutated DNA will generate mRNA that includes the intronic sequence,

which contains an in-frame stop codon (TGA) 30 bp downstream of the splice junction. Hence a truncated and consequently inactive aromatase protein lacking the heme-binding region would be expected to result.

Body changes and physical examination

During 5 yr of observation, starting from a height of 177 cm, the patient reached a height of 183.5 cm before estrogen treatment. Then his height increased slightly and stopped at 184.5 cm after 6 months of estradiol administration. Measurements of the upper to the lower segment and arm span are summarized in Table 1. Body mass index (BMI) increased in the first phases (phases 1–4), remaining substantially unchanged during following phases. The eunuchoid body habitus was first apparent at the age of 23 yr but rapidly worsened with advancing age (Fig. 1 and Table 1).

Axillary and neck acanthosis nigricans appeared at the end of high-dose testosterone treatment (phase 2), before the occurrence of diabetes mellitus. A skin biopsy showed acanthosis nigricans characterized by epidermal hyperplasia and irregular hyperkeratosis. The acanthosis nigricans improved after 1 yr of estradiol treatment (Fig. 3). Skin tags, which are frequently associated with obesity and diabetes mellitus type 2, were also present in phase 5 (Fig. 3).

Bone x-ray

When the patient was 25 yr old, x-ray film of the hand showed a bone age of 15 yr with unfused metacarpal, phalangeal epiphyses.

Bone age did not show any change until phase 5. Conversely, transdermal estradiol treatment (25 μg twice weekly) was effective in promoting bone maturation after 6 months (Table 1).

BMD

BMD in phase 1 showed a pattern of osteoporosis. BMD increased slightly only at the lumbar spine after 14 months

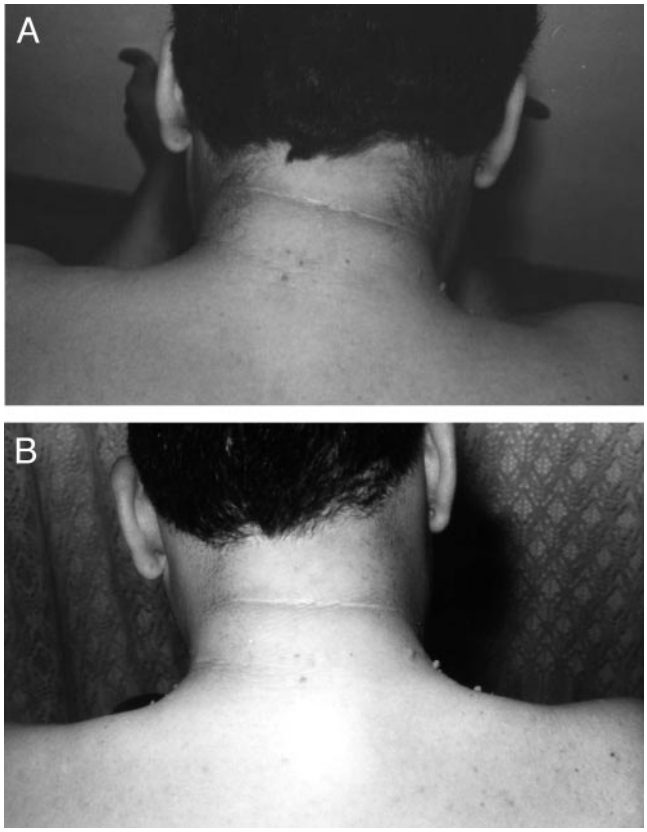

FIG. 3. Neck acanthosis nigricans and skin tags before (A) and after (B) 1 yr of estradiol treatment.

TABLE 1. Anthropometrical and bone parameters

	Phase 1 (baseline)	Phase 2 (TT)	Phase 3 (AT)	Phase 4 (TT)	Phase 5 (BET)	Phase 6 (TE) (25 μg twice weekly)	Phase 7 (TE) (50 μg twice weekly)
Height (cm)	177	179	180	182.5	183.5	184.5	184.5
Weight (kg)	79.6	84	89.0	90.5	93.0	91.5	92
BMI (kg/m^2)	25.4	26.2	27.4	27.1	27.7	26.8	27
Upper to the lower segment ratio (mean, 0.92)	0.88	ND	0.86	ND	0.84	ND	0.85
Arm span	179	ND	182	ND	187	ND	187
Chronological age (yr)	25	25–26	26–27	28–29	29	30	30–31
Bone parameters							
Bone age (yr)	15	15	15	15	15	>16	>16
BMD femoral neck (g/cm^2)	0.796	0.798	0.801	0.750	0.777	0.804	0.813
T score	−2.3	−2.2	−2.1	−2.4	−2.2	−2.0	−1.9
BMD (L2–L4) (g/cm^2)	0.843	0.911	0.910	0.931	0.945	1.015	1.018
T score	−3.3	−2.7	−2.7	−2.6	−2.4	−1.8	−1.8
Calcium (mg/dl) (8.5–10 mg/dl)	9.3	ND	ND	8.8	9.1	9.46	9.49
Phosphorus (mg/dl) (2.5–4.8 mg/dl)	4.1	ND	ND	ND	4.5	4.1	5.8
PTH (pg/ml) (10–65 pg/ml)	ND	ND	ND	ND	18	30	45
Bone-specific alkaline phosphatase (U/liter) (35–120 U/liter)	ND	ND	ND	ND	513	433	407
Osteocalcin (3.1–13.7)	ND	ND	ND	ND	24	9.7	4.4
Urinary D-pyridinium (2.3–5.4 nmol D-Pyr/mmol creatinine)	ND	ND	ND	9.9	9.9	6.87	4.5

To convert values for calcium to millimoles per liter, multiply by 0.2495; to convert values for phosphorus to millimoles per liter, multiply by 0.3229. ND, Not determined; TT, testosterone treatment; AT, alendronate treatment; BET, before estradiol treatment; TE, transdermal estradiol.

of treatment with testosterone enanthate (phase 2) and was not modified by both 10 months of alendronate (phase 3) and subsequent testosterone treatments (phase 4) (Table 1).

After 6 months of estradiol treatment (25 μg twice weekly), BMD increased substantially (Table 1), reaching values that were maintained during the following 6 months of treatment with 50 μg estradiol twice weekly.

Pituitary-testicular axis

In phase 1, LH concentrations were in the normal range, whereas FSH levels were increased and serum testosterone was at the lower limit of the normal range (Table 2). High-dose testosterone treatment restored normal to high circulating levels of testosterone in phases 2 and 4. Despite normal to high serum testosterone in phase 4, serum estradiol was below the assay detection limit (<1.5 pg/ml). Before estradiol treatment (phase 5), serum testosterone levels were slightly below the normal range; LH was normal with high serum levels of FSH. Estradiol treatment resulted in a decrease in LH, FSH, and testosterone serum levels, which was inversely related to the dose administered, except for FSH, whose values did not change when the dosage of estradiol was increased (phase 7). Prolactin levels remained unchanged during all phases (Table 2).

A biopsy of the testis, performed 8 months after the withdrawal of the second testosterone treatment (phase 5), revealed a pattern of total germ cell depletion (Fig. 4). Because the patient refused to undergo semen analysis, a sperm count was not available.

Glucose metabolism

Serum glucose concentrations were in the normal range in phases 1, 2, and 3. In phase 1 serum insulin was only just above the upper limit of the normal range (Table 3). During

TABLE 2. Sex steroids and gonadotropin serum levels

	Phase 1 (baseline)	Phase 2 (TT)	Phase 3 (AT)	Phase 4 (TT)	Phase 5 (BET)	Phase 6 (TE, 25 μg twice weekly)	Phase 7 (TE, 50 μg twice weekly)
Pituitary-testicular axis							
LH (1.5–9.2 mIU/ml)	3.7	ND	ND	ND	7	5.9	4.29
FSH (1.2–7.8 mIU/ml)	23	ND	ND	ND	20	10.5	11.54
Testosterone (3.0–9.0 ng/ml)	3.8	7.2	2.1	9.1	2.7	1.45	0.8
Estradiol (30–90 pg/ml)				<1.5	<1.5	23.5	41
Prolactin (2–18 ng/ml)	4.6	3.8	4.2	3.7	3.8	4.2	4.0

To convert values for testosterone to nanomoles per liter, multiply by 0.035; to convert values for estradiol to picomoles per liter, multiply by 3.671. ND, Not determined; TT, testosterone treatment; AT, alendronate treatment; BET, before estradiol treatment; TE, transdermal estradiol.

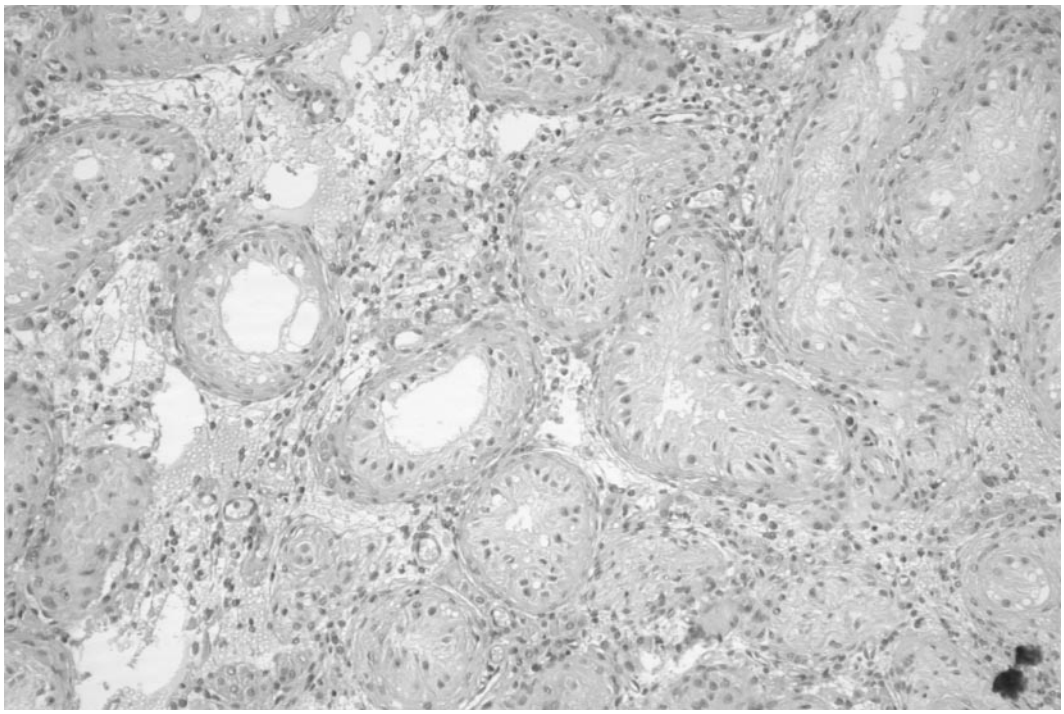

FIG. 4. Biopsy of the testis shows hypotrophic seminiferous tubules together with mature Sertoli cells with abundant eosinophilic cytoplasm. A total germ cell depletion without any spermatogenic development was evident. Leydig cells were of the mature type and organized in scarce small groups. In addition, vascular and connective tissue interstitial fibrosis together with mononuclear infiltration was also present. The histopathological diagnosis was total germ cell depletion (Sertoli cells only syndrome) with focal schlerohyalinosis of seminiferous tubules.

TABLE 3. Lipids, glucose, insulin serum levels, and liver function

	Phase 1 (baseline)	Phase 2 (TT)	Phase 3 (AT)	Phase 4 (TT)	Phase 5 (BET)	Phase 6 (TE, 25 μg twice weekly)	Phase 7 (TE, 50 μg twice weekly)
Metabolic parameters							
Total cholesterol (<200 mg/dl)	180	199	220	183	177	182	110
LDL cholesterol (<130 mg/dl)	ND	ND	144	140	107.25	119	66
HDL cholesterol (>40 mg/dl)	ND	ND	33	28	31	37	41
Triglycerides (<200 mg/dl)	ND	ND	144	209	199	148	106
Glucose (70–110 mg/dl)	74	82	85	154	180	156	144
Insulin (5–30 μU/ml)	31	56	45	112	94	75	53
HbA1c	ND	ND	ND	ND	8.3%	8.1%	7.6%
Fructosamine (0.285 μmol/liter)	ND	ND	ND	ND	406	317	315
Liver function parameters							
GPT (<37 U/liter)	225	218	210	165	195	125	70
GOT (<40 U/liter)	188	104	130	96	108	86	45
γ-GT (11–50 U/liter)	ND	ND	ND	ND	153	95	42
Alkaline Phosphatase (98–279 U/L)	ND	ND	ND	ND	687	728	639

To convert values for total cholesterol, HDL cholesterol, and LDL cholesterol to millimoles per liter, multiply by 0.025; to convert values for triglycerides to millimoles per liter, multiply by 0.011; to convert values for glucose to millimoles per liter, multiply by 0.055. ND, Not determined; TT, testosterone treatment; AT, alendronate treatment; BET, before estradiol treatment; TE, transdermal estradiol.

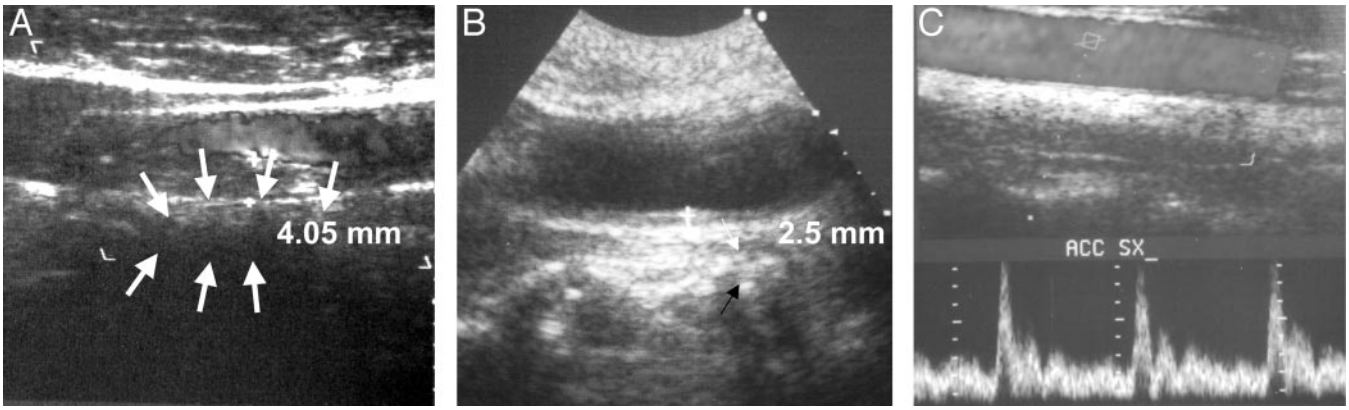

FIG. 5. Echo-Doppler outcome. A, A lipid plaque of 4 mm (*white arrows*) in the left common carotid. B, Reduction of plaque size (2.5 mm) after 6 months of transdermal estradiol treatment (*white* and *black arrows*). C, In phase 7 the carotid plaques disappeared.

phase 2 axillary and neck acanthosis nigricans occurred (Fig. 3) together with an increase in serum insulin levels. In phase 4, during the second testosterone treatment, serum glucose was elevated, consistent with a diagnosis of diabetes mellitus together with a further increase of serum insulin (Table 3). The assay of islet cell autoantibodies, antiinsulin autoantibodies, and C-peptide excluded the diagnosis of type 1 diabetes (data not shown).

Glucose, insulin, HbA1c, and fructosamine serum levels decreased and acanthosis nigricans clinically improved during transdermal estradiol treatment according to the dosage used (Table 3 and Fig. 3B).

Lipid metabolism and vascular outcomes

During the second testosterone treatment (phase 4), serum triglycerides were elevated and HDL cholesterol was decreased, whereas total and LDL cholesterol remained unchanged. Serum triglycerides and serum total and LDL cholesterol were in the normal range before transdermal estradiol treatment (phase 5), and they progressively decreased with a concomitant increase of HDL cholesterol reaching normal values during estradiol treatment (Table 3).

An echo-Doppler study of carotid arteries in phase 5 at the patient's age of 30 yr showed two lipid plaques in the left common and internal carotid of 4 and 3 mm of thickness, respectively. Transdermal estradiol treatment resulted in a reduction of plaques sizes after 6 months of treatment (2.5 and 2 mm, respectively) and subsequently in a complete disappearance of the plaques (Fig. 5).

Liver metabolism

Liver function was affected since the first observation (phase 1) with increased values of hepatic enzymes (Table 3). Laboratory analysis excluded infections from virus hepatitis A, B, and C as well as other causes of hepatitis. A liver biopsy performed in phase 5 showed a pattern of nonalcoholic steatohepatitis (NASH) (Fig. 6A). Estrogen administration resulted in a progressive improvement of circulating GOT, GPT, and γ-GT. Consistent with this finding, liver biopsy performed during estradiol treatment showed an improvement of liver NASH (Fig. 6B).

Side effects

Estrogen treatment did not result in any noticeable side effect. The patient did not develop gynecomastia.

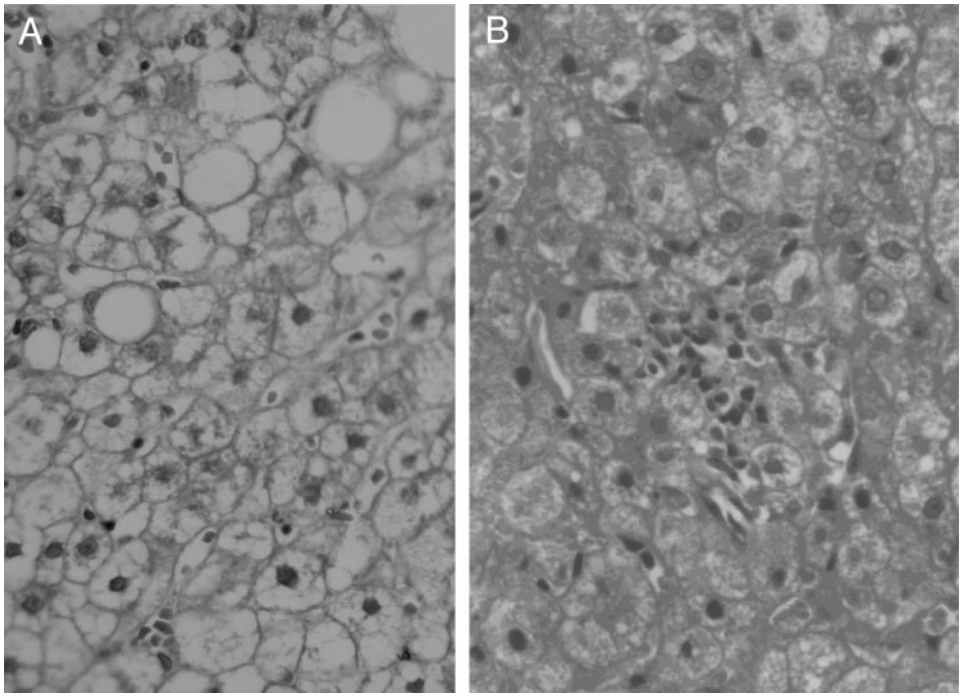

FIG. 6. A, Liver biopsy, performed in phase 5, is characterized by macro- and microsteatosis together with periportal fibrosis showing a pattern of NASH. B, Liver biopsy, performed in phase 7, shows an evident improvement of the liver macro- and microsteatosis after 1 yr of transdermal estradiol treatment.

Discussion

In this paper we describe a new case of a homozygous mutation of the aromatase gene in a man resulting in congenital estrogen deficiency. The propositum presented with lack of skeletal maturation, impairment of testicular function, and a complex dysmetabolic syndrome. The analysis of the effects induced by the successive treatment of this patient with a high dose of testosterone, alendronate, and estradiol allows further insight into the roles of androgens and estrogens regarding several metabolic functions.

Bone maturation and BMD

A long-term, high-dose testosterone treatment performed for more than 2 yr at different dosages resulting in physiological to supraphysiological serum testosterone levels was ineffective in improving bone maturation, whereas it resulted in a slightly increase of bone mineralization only at the lumbar spine, thus confirming results from a previous patient after 6 months of treatment (5). Estrogen treatment resulted in an increase in BMD both at lumbar spine and femoral neck. Thus, in men epiphyseal closure and BMD are regulated by estrogens rather than androgens (3–12). These clinical outcomes are consistent with the recent progress made in understanding the mechanisms of estrogen action on the growth plate (1, 19).

Recently, alendronate (an inhibitor of the osteoclast-mediated bone resorption) proved to be effective in increasing BMD in men with osteoporosis, an effect that was independent of baseline free testosterone or estradiol concentrations (20). By contrast, in this aromatase-deficient man, 10 months of alendronate treatment was completely ineffective. Alendronate acts by inhibiting bone resorption, and it is more effective in osteoporosis when enhanced bone resorption occurs. Even though 10 months of treatment might not be sufficient to evaluate the effectiveness of alendronate therapy, the relative failure of the treatment here documented is probably due to inability of the patient to reach the peak of bone mass, thus supporting the hypothesis that in congenital estrogen deficiency osteoporosis is mostly the result of a lower peak bone mass (21), rather than an increased bone resorption. Although 10 months of alendronate treatment is a relatively short period of treatment, in men a positive effect on BMD is often already evident after 6 months (20). In addition, estrogen could act as a permissive factor for bisphosphonate action because the concomitant use of both estrogen and alendronate treatment was more effective than the separate use of these two drugs (22).

It has to be noted that both epiphyseal closure and increase in BMD occurred during estrogen treatment when serum testosterone was very low. Thus, the concept that estrogen is the principal sex steroid involved in the final phases of skeletal maturation and mineralization is reinforced. Accordingly, eunuchoid body proportions of the skeleton are strongly associated with congenital estrogen deficiency in men (3–9). The case presented here is anecdotal in demonstrating that this phenomenon is the result of estrogen deficiency rather than androgen deprivation because it worsened after puberty (Table 1 and Fig. 1), despite androgen supraphysiological supplementation (phases 1–5 and Table 2). Finally, these data support the concept that this mechanism operates also in men with hypogonadism and/or delayed puberty, in which both eunuchoid body proportions and osteopenia are the result of relative estrogen deficiency due to insufficient availability of androgen for aromatization to estrogen.

Pituitary-testicular axis

At baseline, basal serum LH was normal and basal serum FSH was clearly above the normal range. In fact, basal serum FSH was higher than normal in all the four adult men with aromatase deficiency (4–8, 13, 23), whereas basal serum LH was above the normal range in only one patient (4, 6), at the upper limit of the normal range in another patient (5, 7, 13), and normal in the remaining two patients (8, 23). Estrogen treatment resulted in a decrease of both LH and FSH basal serum levels but with a more evident effect on FSH. Even though the study is limited by the evaluation of a simple basal serum sample, the results confirm that LH and particularly FSH depend on circulating estrogen (21) as in other aromatase-deficient men (5–8, 13). Consistent with this, studies based on the evaluation of estrogen suppression in normal males demonstrated that testosterone exerts a negative control on LH secretion by acting directly or indirectly (after the conversion into estrogens) (14, 15), whereas the effects on FSH are mainly mediated by aromatization to estrogens (24).

Even though the biopsy of the testis alone is not adequate for the diagnosis of male infertility, because germ cells may be present in other part of the testis, the histological appearance suggests that patient's fertility is impaired. Because in this case there is no semen analysis to provide evidence on the degree of fertility impairment, a comparison with previously described aromatase-deficient men (4–8), in which the degree of fertility failure varies widely (23), is not possible. However, if cryptorchidism and the volume and soft consistency of the testes together with their position in the inguinal canal and the impairment of androgen secretion (particularly in phases 5, 6, and 7) are all taken into account, it seems that testicular failure is more severe in this patient than in other aromatase-deficient men (4–8), and all these events may account for the disruption of spermatogenesis. Regarding the cryptorchidism, recently a history of left orchidopexy has also been described in a 25-yr-old man with aromatase deficiency and asthenozoospermia (25). The etiology of testicular maldescent is not completely clear, but it is frequent in patients with a deficit of both circulating estrogen and testosterone during fetal development (26). In rodents a possible role of estrogen receptor-α in testicular descent was suggested (27), but aromatase knockout (ArKO) male mice have normal testicular descent (2). In conclusion, an impairment of fertility is often present in men affected by congenital aromatase deficiency (3–8, 25). Studies performed on estrogen receptor-knockout and ArKO male mice have demonstrated a major role of estrogens in male fertility in rodents (2), but further studies are needed to elucidate the real impact of estrogen on human reproduction.

Glucose metabolism

The patient developed bilateral axillary and neck acanthosis nigricans in phase 2 and diabetes mellitus type 2 during phase 4 (both during high-dose testosterone treatment) together with a concomitant slight increase in BMI. The restoration of normal serum estrogen resulted in a progressive reduction of the acanthosis nigricans and an improvement of glycemic control in both phases 6 and 7. Also, estrogen treatment resulted in reduced basal serum insulin levels in two other aromatase-deficient men (5, 6), and acanthosis nigricans was also present in the estrogen-resistant man together with increased fasting glucose (3). Notwithstanding normal plasma glucose during an oral glucose tolerance test, insulin resistance has been recently demonstrated in a 27-yr-old aromatase-deficient man by means of elevated homeostasis model assessment of insulin resistance index. In this patient, however, estrogen treatment was unable to modify insulin resistance (homeostasis model assessment of insulin resistance), but it decreased the area under the curve of glucose, insulin, and C-peptide levels and serum testosterone (8). These results suggest a probable role of sex steroids in insulin sensitivity and the development of insulin resistance.

In the present case, the long period of treatment with supraphysiological doses of testosterone (phases 2 and 4) should be taken into account because it reduced estradiol to testosterone ratio and worsened insulin resistance and glucose metabolism, but a direct cause-effect relationship between elevated testosterone and insulin resistance cannot be demonstrated. In normal men supraphysiological doses of exogenous testosterone do not affect insulin sensitivity, but a concomitant increase of serum estradiol occurs due to a normally functioning aromatase enzyme (28), so that the estradiol to testosterone ratio remains unchanged. Conversely, in our patient insulin resistance and diabetes mellitus type 2 occurred during high-dose androgen treatment coupled with the congenital lack of estrogens, suggesting an involvement of both these conditions in this process. Data from rodents speak in favor of this hypothesis because impaired glucose tolerance and insulin resistance are accompanied by both the lack of estrogen and moderate hypertestosteronemia in ArKO and estrogen receptor-knockout mice (2, 29, 30). Thus, this case provides further evidence for a role of unopposed androgen action in glucose metabolism, suggesting that a severe impairment of the estrogen to testosterone ratio could represent a condition of high risk for the development of insulin resistance in men, as previously suggested by Grumbach and Auchus (21). Also, weight gain might have contributed to the development of diabetes mellitus type 2, even though the concomitant increase of the patient's height did not result in a substantially increase of BMI.

In addition, a direct positive effect of estrogen treatment on insulin sensitivity needs to be pointed out because estradiol was able to reverse, at least in part, insulin resistance and improve the control of diabetes mellitus type 2. This result confirms a direct involvement of estrogen in the control of glucose metabolism in men, as previously suggested from the study of other aromatase-deficient men (4–9, 23) and emphasizes that high-serum testosterone may affect insulin sensitivity when estrogen activity is absent (21).

Moreover, the mechanism by which the lack of estrogen may induce insulin resistance in this patient is complicated by other concomitant diseases. First of all, NASH and insulin resistance are often associated, and even though the nature of their interconnection is still unknown, NASH may have contributed to the development of insulin resistance in this patient (31). Again, the occurrence of type 2 diabetes mellitus constitutes a confounding factor in interpreting data on the effects of estradiol treatment on glucose and insulin metab-

olism in this patient. Notwithstanding, estradiol treatment also improved fasting serum glucose, HbA1c, and fructosamine, suggesting a possible positive role of estrogens on glucose metabolism, which is supported by unchanged BMI and the finding of a normal lipid profile during estradiol treatment.

In conclusion, abnormalities in the estradiol to testosterone ratio (increased androgens and decreased estrogens) could lead to insulin resistance that may be reversed by estrogen treatment through a direct positive effect on glucose metabolism and a restoration of normal estradiol to testosterone ratio. This concept may be helpful for a better comprehension of both the pathogenesis and treatment of various dysmetabolic disorders, but the mechanisms by which sex steroids affect carbohydrates metabolism in men remains poorly understood.

Lipid metabolism and vascular outcomes

Supraphysiological doses of testosterone in phase 4 decreased HDL cholesterol without change in BMI but with concomitant marked increase in serum insulin. HDL cholesterol is inversely related to serum testosterone (28, 32, 33), but in this case also hyperinsulinism may have contributed to the decrease in HDL cholesterol. Conversely, estradiol treatment resulted in a moderate increase in HDL cholesterol coupled with a slight decrease in triglycerides and total and LDL cholesterol, an effect that has been previously shown in the other two aromatase-deficient men (4–7) as well as in the ArKO mice (30). A decrease of insulin serum levels that occurred during the transdermal estradiol treatment may have also contributed to improve circulating HDL cholesterol and triglycerides.

The finding of carotid atherosclerosis in a very young man is not a common clinical outcome, but a precocious alteration of coronary morphology and function was demonstrated also in the young estrogen-resistant man (34, 35). Estradiol treatment resulted in size reduction and disappearance of carotid atherosclerosis in the present patient, thus emphasizing a role for estrogens on cardiovascular function in men. Recently it has been suggested that estrogen may prevent the progression toward calcification at the plaque site (36). Estrogens have a positive role on the male cardiovascular system and survival (37, 38), and their supplementation in men may have a possible beneficial effect, especially during senescence (39). Thus, the coexistence of endothelial dysfunction (35, 40) early atherosclerosis and an abnormal lipid pattern, which is related to estrogen deficiency in men (21), implies that men with congenital aromatase deficiency have a high risk of cardiovascular disease at a young age.

Liver metabolism

On the basis of liver enzyme levels and liver biopsy, the diagnosis of NASH was made in this patient. NASH was present in phase 1 and preceded the occurrence of diabetes mellitus and probably of insulin resistance, too. However, even though signs of insulin resistance appeared only in phase 2, standing the high serum insulin levels in phase 1, we may not exclude that insulin resistance was present together with NASH since baseline. Notwithstanding insulin resistance and diabetes mellitus, which usually worsen liver function, estradiol treatment resulted in an improvement of both liver enzymes and morphology. It is difficult to separate the possible beneficial effect of estrogen treatment from other confounding variables because the improvement in both diabetes mellitus and insulin resistance may account for liver function and structure amelioration. An association between insulin resistance and NASH is, in fact, well known (31). Moreover, the ArKO mouse displays insulin resistance and liver steatosis (30, 41) and thus resembles the aromatase-deficient man in this context also. Furthermore, estrogen supplementation may improve hepatic function in women with Turner's syndrome and hypoestrogenism (42), whereas treatment with aromatase inhibitors for male infertility may increase serum liver enzymes (43). Lastly, a cohort of Japanese women treated with tamoxifen has been reported to develop hepatic steatosis (44). Taken together, these findings suggest a possible involvement of estrogen deficiency in the development of liver steatohepatitis in this patient.

Conclusions

This new case of aromatase deficiency reveals a relationship between estrogen deficiency and the occurrence of a dysmetabolic syndrome consisting of altered glucose and liver metabolism coupled with precocious atherogenesis and acanthosis nigricans. Thus, it reveals new and hitherto unrecognized actions of estrogens on metabolic parameters in the male, which imply that aromatase-deficient men may be at higher risk for the development of cardiovascular disease and emphasizes the need of an adequate clinical follow-up. These findings emphasize a growing repertoire of actions of estrogens and androgens that are nonsexually dimorphic and are unrelated to reproduction.

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