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Estrogen Suppression in Males: Metabolic Effects*

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ABSTRACT

We have shown that testosterone (T) deficiency *per se* is associated with marked catabolic effects on protein, calcium metabolism, and body composition in men independent of changes in GH or insulin-like growth factor I production. It is not clear, however, whether estrogens have a major role in whole body anabolism in males. We investigated the metabolic effects of selective estrogen suppression in the male using a potent aromatase inhibitor, Arimidex (Anastrozole). First, a dose-response study of 12 males (mean age, 16.1 ± 0.3 yr) was conducted, and blood withdrawn at baseline and after 10 days of oral Arimidex given as two different doses (either 0.5 or 1 mg) in random order with a 14-day washout in between. A sensitive estradiol (E_2) assay showed an approximately 50% decrease in E_2 concentrations with either of the two doses; hence, a 1-mg dose was selected for other studies. Subsequently, eight males (aged 15–22 yr; four adults and four late pubertal) had isotopic infusions of [^{13}C]leucine and ^{42}Ca/^{44}Ca, indirect calorimetry, dual energy x-ray absorptiometry, isokinetic dynamometry, and growth factors measurements performed before and after 10 weeks of daily doses of Arimidex. Contrary to the effects of T withdrawal, there were no significant changes in body composition (body mass index, fat mass, and fat-free mass) after estrogen suppression or in rates of protein synthesis or degradation; carbohydrate, lipid, or protein oxidation; muscle strength; calcium kinetics; or bone growth factors concentrations. However, E_2 concentrations decreased 48% ($P = 0.006$), with no significant change in mean and peak GH concentrations, but with an 18% decrease in plasma insulin-like growth factor I concentrations. There was a 58% increase in serum T ($P = 0.0001$), sex hormone-binding globulin did not change, whereas LH and FSH concentrations increased ($P < 0.02$, both). Serum bone markers, osteocalcin and bone alkaline phosphatase concentrations, and rates of bone calcium deposition and resorption did not change. In conclusion, these data suggest that in the male 1) estrogens do not contribute significantly to the changes in body composition and protein synthesis observed with changing androgen levels; 2) estrogen is a main regulator of the gonadal-pituitary feedback for the gonadotropin axis; and 3) this level of aromatase inhibition does not negatively impact either kinetically measured rates of bone calcium turnover or indirect markers of bone calcium turnover, at least in the short term. Further studies will provide valuable information on whether timed aromatase inhibition can be useful in increasing the height potential of pubertal boys with profound growth retardation without the confounding negative effects of gonadal androgen suppression. (*J Clin Endocrinol Metab* **85:** 2370–2377, 2000)

THERE IS A sexually dimorphic pattern of the effects of gonadal steroids on the neuroendocrine axis *vs.* their metabolic effects. Studies performed in young adults and prepubertal boys have shown that both aromatizable and nonaromatizable androgen significantly increase measures of whole body protein synthesis (1, 2) and mixed muscle protein synthesis (3) in humans. Estrogen administration to young hypogonadal girls, however, was not associated with any measurable change in whole body protein kinetics (4), suggesting that androgens may have a direct effect on whole body protein pools and not via aromatization. As it pertains to its impact on GH production, however, testosterone appears to increase GH production rates in childhood indirectly. When a nonaromatizable androgen such as dihydrotestosterone is given, the increase in GH production observed after testosterone treatment is no longer observed (5), and when tamoxifen, an estrogen receptor blocker, is given to pubertal children, GH production is decreased (6). These data suggest that as it pertains to its central effect, androgens affect GH production mostly through aromatization to estrogens. The importance of estrogen's effects in males is further evidenced by the reports of two cases, one of an adult, well virilized male, still growing past puberty, identified as having a point mutation in the estrogen receptor gene (7), and the other of a male with a mutation in the aromatase enzyme (8, 9). These data strongly suggest that even in the male, estrogen may be the most important hormone involved in epiphyseal closure. Additional support for this concept was obtained when a new estrogen receptor blocker was given to estrogen-treated mice, blocking the acceleration in bone maturation caused by estrogen (10).

Children with treatable forms of severe growth retardation presenting in puberty have the disadvantage of a limited time available for growth-promoting agents to work. Hence, GnRH analogs (GnRHa) have been commonly used to suppress puberty and delay epiphyseal fusion in this situation. Based on the knowledge gained from aromatase- and estrogen-deficient models, a better strategy to maximize height potential in very short males treated with recombinant human GH might be to suppress estrogen production while allowing complete virilization. This would offer potentially many advantages over GnRHa therapy.

Two recent developments now allow assessment of the effects of selective estrogen suppression in males under con-

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trolled experimental conditions. First is the availability of Arimidex (anastrozole, AstraZeneca, Wilmington, DE); this is a novel, nonsteroidal aromatase inhibitor [1,3-benzenediacetonitrile, $\alpha,\alpha,\alpha',\alpha'$-tetramethyl-5-(1H-1, 2, 4-triazol-1-ylmethyl)] that blocks the conversion of Δ^4-androstenedione to estrone and of testosterone to estradiol. It is metabolized mostly in the liver (85%), with an elimination half-life of 50 h and a terminal elimination half-life of 2 days. Plasma concentrations reach steady state levels at about 7 days of once daily treatment. Extensive data generated in both animals and humans indicate that this compound has no other pharmacological effect *in vivo* (11–13); it is presently an FDA-approved drug for the treatment of metastatic breast cancer (14). Secondly, the availability of a highly sensitive estrogen assay allows the measurement of circulating estradiol (E_2) concentrations at levels below those detectable by conventional assays (15).

Hence, we designed these studies with the following specific questions. 1) What dose of anastrozole can be safely given in adolescent youngsters with minimal or no side-effects that maximally suppresses E_2 concentrations? 2) Does anastrozole administration affect measures of whole body protein and bone calcium metabolism and GH production in males? To accomplish this, a group of young eugonadal males, in both late puberty and young adulthood, were recruited. Specific measures of intermediate metabolism were assessed before and after the administration of anastrozole.

Materials and Methods

Study design

All studies were approved by the Nemours clinical research review committee and Wolfson Children's Hospital institutional review board. The study drug was administered under the principal investigator's (N.M.) own investigational new drug number assigned by the FDA.

Study 1 (question 1)

A group of 12 healthy young male volunteers (14–18 yr old; Tanner stage IV–V) was recruited after informed written consent was given. These subjects were given anastrozole orally at 2 different doses (0.5 or 1 mg) for 10 days, in random order, with 14 days of washout in between to allow approximately 7 half-lives of elimination of the drug. Blood was withdrawn in the early morning pre- and posttreatment for the measurement of serum testosterone, ultrasensitive E_2, LH, FSH, dehydroepiandrosterone sulfate (DHEAS), and insulin-like growth factor I (IGF-I) concentrations. Liver function tests and blood counts were monitored also.

Study 2 (question 2)

Eight healthy male volunteers, aged 15–22 yr, were recruited after giving informed written consent. The subjects who were still growing were at least Tanner stage IV–V of genital development.

Experimental design

Before admission to the Wolfson Children's Hospital clinical research center (CRC), subjects were encouraged to consume a weight maintenance diet for 3 days, consisting of approximately 35 Cal/kg and 1.5 g/kg protein·day. Dual emission x-ray absorptiometry (DEXA), using a tissue bar (Hologic 2000, Hologic, Inc., Waltham, MA) was used for assessment of bone mineral density and body composition; skinfold calipers and bioelectrical impedance analysis were also used . Isokinetic and isometric dynamometry of the anterior quadriceps was performed in our physical therapy department using a Biodex dynamometer (Biodex Corp., Shirley, NY). After a 10-min training session and 30 min of rest, maximum work and torque measurements for isometric and isokinetic tests were made. Isometric tests were performed with 5 contractions of 5 s each with the knee placed at 45° of flexion, with 10 s of rest between contractions. Isokinetic tests were performed for knee extension and flexion at 60°/s for 5 repetitions and at 180°/s for 21 repetitions as described previously (1).

The evening before admission to the CRC, after eating dinner at 1800 h, subjects were fasted, except for water *ad libitum*, until the completion of the studies at 1300 h the next day. The following morning (baseline study), the subjects were admitted to the CRC, and at 0600 h, two iv needles were placed in each antecubital vein; one was kept heated for arterialized blood sampling (16). At 0800 h (time zero) a primed, dose constant infusion of L-[1-^{13}C]leucine (4.5 μmol/kg; 0.07 μmol/kg·min) was begun and was continued uninterrupted for the next 240 min. At time zero, 0.15 mg/kg ^{42}Ca was also given as a slow iv push over 5 min. Frequent blood draws were performed as detailed below, and a urine collection was begun and continued for the next 28 h. Frequent breath samples were also obtained for determination of $^{13}CO_2$ enrichment in expired breath. Using a mouthpiece, indirect calorimetry was performed three times during the 4 h of isotope infusion using a CPX max indirect calorimeter (Medical Graphics, St. Paul, MN) After the iv isotope infusions were completed at 1200 h, 0.3 mg/kg of a stable isotope of calcium (^{44}Ca) was given orally mixed with juice (prepared at least 12 h before equilibration), and the patients were fed lunch. Subsequently, one iv line was discontinued, and subjects were free to move around until 1600 h, when a final blood sample was obtained for determination of Ca enrichments. Subjects were then sent home to complete the urine collection, and twice daily urine samples were obtained for determination of the Ca isotopic enrichments for the next 5 days.

After the baseline study and all urine collections were completed, patients were started on Arimidex at a dose of 1 mg given orally daily. Blood was withdrawn 1 and 4 weeks after the initiation of the treatment for determination of hormone concentrations and safety analysis (blood counts, urinalysis, and liver profiles). Ten weeks after the baseline study an identical study was carried out (D2).

Blood and breath samples

The isotopic enrichments of α-ketoisocaproic acid (^{13}C labeled) were measured at −20, 160, 180, 200, 220, and 240 min. The Ca isotopic enrichments were measured at 0, 5, 10, 15, 20, 30, 40, 60, 120, 180, 240, and 480 min. Plasma IGF-I, IGF-binding protein-3 (IGFBP-3), testosterone, free testosterone, insulin, and glucose concentrations were measured three times during the 240 min of tracer infusions. Serum GH concentrations were measured at 10-min intervals for the 4 h of the studies. Serum lipids were also measured while subjects were fasting on each study day. Breath samples were obtained for the measurement of expired labeled CO_2 at −20, −10, −5, 160, 180, 200, and 220 min. A small aliquot of the urine collected during the 4-h morning study was used for determination of the urea nitrogen concentration.

Assays

Plasma enrichments of [^{13}C]α-ketoisocaproic acid were determined at the Nemours metabolic core laboratory by gas chromatography mass spectrometry (17, 18) and $^{13}CO_2$ by isotope ratio mass spectrometry as described previously (19). Urinary Ca was determined by flame atomic absorption spectrophotometry at the laboratory of Dr. O'Brien. A dual filament thermal ionization quadrapole mass spectrometer (Finnigan THQ, Finnigan MAT, Bremen, Germany) was used to measure the Ca isotopic enrichments (20). Insulin was measured by an immunoenzymatic method (Sanofi Pharmaceuticals, Inc., Chaska, MN), testosterone by a chemiluminescence assay (Chiron Corp., East Walpole, MA), free testosterone by RIA (Diagnostic Systems Laboratories, Inc., Webster, TX), DHEAS by chemiluminescence (Diagnostic Products, Los Angeles, CA), bone-specific alkaline phosphatase by an immunoassay using a monoclonal antibody (Metra Biosystems, Mountain View, CA), and osteocalcin by an immunoradiometric assay (Oris Group, Gif-Sur-Yvette, France). IGF-1, IGFBP-1, IGFBP-2, and IGFBP-3 were measured by immunoradiometric assays at the Mayo Clinic immunochemical core laboratory (Rochester, MN). A sensitive chemiluminescence assay was used to measure GH concentrations (21); LH and FSH were measured by RIA, all at the University of Virginia General Clincial Research Center

core laboratory. A highly sensitive recombinant cell bioassay was used to measure E_2 concentrations with a sensitivity of 0.2 pg/mL (0.73 pmol/L) (15). Urea nitrogen was measured using a Kodak Ektakem urease method (Eastman Kodak Co., Rochester, NY).

Calculations

Estimates of rates of whole body protein turnover at steady state were calculated using the reciprocal pool model as previously described (17, 18). The fractional Ca absorption (α) was calculated from the ratio of the cumulative excretion of the oral tracer (^{44}Ca) in urine divided by the cumulative excretion of the iv tracer (^{42}Ca) as previously described (22, 23). True Ca absorption is determined by the formula $Va = Vi \times \alpha$, where Vi is the dietary Ca intake. Ca kinetic analysis was performed by measuring the isotopic enrichments of the Ca tracers in blood and urine over time using a multicompartmental model and the simulation analysis and modeling program, SAAM, as previously described (22, 24, 25). Substrate oxidation rates for protein, glucose, and lipid and resting energy expenditure were calculated using the rate of gas exchange (VO_2 and VCO_2) from the indirect calorimetry as previously described (26). Fat-free mass and percentage of fat mass were measured using DEXA and the tissue bar as well as by the sum of skin folds as described previously (27).

Data for changes in rates of leucine kinetics, body composition, and measures of muscle strength in the Arimidex-treated subjects were compared with the changes observed in eight GnRHa-treated males (aged, 18–25 yr) treated with the GnRHa Lupron for two doses of 7.5 mg, given 4 weeks apart. These subjects were studied identically as the present Arimidex-treated cohort, twice, 10 weeks apart, and some of their data have been reported previously (1, 28).

Isotopes and drugs

L-[1-^{13}C]Leucine (99% enriched; Cambridge Isotopes, Andover, MA) and the $^{42}Ca/^{44}Ca$ ratio (93.5% and 96% enriched, respectively; Trace Sciences International, Richmond Hill, Canada) were determined to be sterile and pyrogen free and were mixed with 0.9% nonbacteriostatic saline. Arimidex (AstraZeneca Pharmaceuticals, Wilmington, DE) was bought commercially in 1-mg tablets.

Statistical analysis

Results are expressed as the mean ± SE. Paired Student's t test was used to estimate differences between both study days. ANOVA was used to estimate the differences between Arimidex- *vs.* Lupron-treated subjects. Significance was established at $P < 0.05$.

Results

Protocol 1

Table 1 summarizes the changes in circulating hormone concentrations after anastrozole administration. Both doses were associated with comparable suppression of E_2 (~50%), with parallel increases in testosterone and free testosterone concentrations without any change in IGF-I (Fig. 1). Three subjects also received 3 mg Arimidex in an identical paradigm, yet there was a similar percent decline in E_2 concentrations and a reciprocal increase in testosterone concentrations as with the 0.5- and 1-mg doses (data not shown). As all doses worked comparably in suppressing estrogen concentrations, and the tablet is compounded as 1 mg, we chose to use 1 mg as the dose used for all subsequent studies.

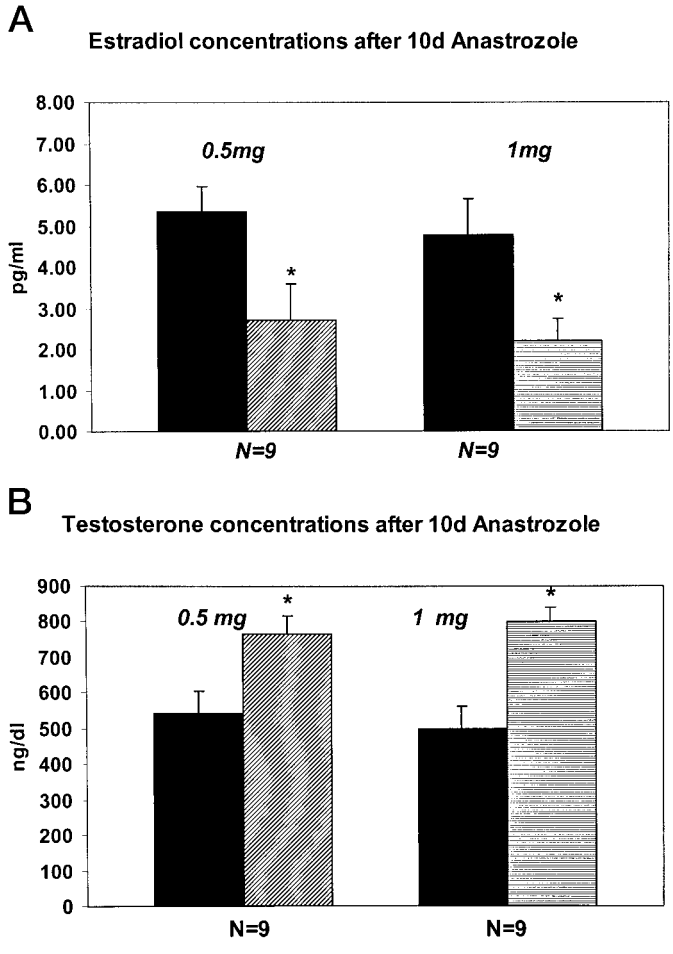

FIG. 1. Changes in testosterone and E_2 concentrations in normal young men (15–22 yr old) before (■) and after 10 days of oral anastrozole at 0.5 and 1 mg.

TABLE 1. Mean (±SEM) changes in circulating concentrations of different hormones after 10 days of anastrozole orally in healthy boys/young men

	0.5 mg/day (n = 9)				1 mg/d (n = 9)			
	Day 1	Day 2	%Δ	*P*	Day 1	Day 2	%Δ	*P*
Testosterone, ng/dL (nmol/L)	543 ± 62 (18.8 ± 0.2)	766 ± 51 (26.6 ± 0.2)	41	4E-5	499 ± 63 (17.3 ± 0.2)	802 ± 41 (27.8 ± 0.1)	61	1E-4
Free T, pg/mL	15.6 ± 2.2	25.1 ± 2.1	61	1E-4	15.9 ± 1.7	27.1 ± 2.5	70	2E-3
E_2, pg/mL (pmol/L)	5.36 ± 0.80 (19.7 ± 2.9)	2.7 ± 0.62 (9.9 ± 2.3)	−50	5E-4	4.78 ± 0.88 (17.5 ± 3.2)	2.22 ± 0.54 (8.1 ± 2.0)	−54	0.02
SHBG, nmol/L	21.5 ± 2.2	21.9 ± 2.1	1.6	0.77	21.2 ± 2.0	20.0 ± 1.7	−5.6	0.24
IGF-I, ng/mL or μg/L	351 ± 40	326 ± 28	−6.9	0.34	330 ± 31	326 ± 32	−1.3	0.80
DHEAS, ng/mL; μmol/L	2082 ± 311 (5.7 ± 0.8)	2365 ± 273 (6.4 ± 0.7)	14	6E-3	2205 ± 363 (6.0 ± 1.0)	2432 ± 360 (6.6 ± 1.0)	10	0.04

SI units are given in *parentheses*. %Δ, Percent change.

TABLE 2. Mean (±SEM) changes in hormones, growth factors, and substrates in young men treated with 1 mg anastrozole, orally, daily for 10 weeks

	Day 1	Day 2	*P* value
Testosterone, ng/dL (nmol/L)	522 ± 72 (18.1 ± 0.2)	822 ± 41 (28.5 ± 0.1)	0.0001
Free testosterone, pg/mL	20 ± 2	32 ± 2	0.0005
Estradiol, pg/mL (pmol/L)	8.06 ± 0.81 (29.6 ± 3.0)	4.22 ± 1.09 (15.5 ± 4.0)	0.006
DHEAS, ng/mL (μmol/L)	2373 ± 431 (6.4 ± 1.1)	2303 ± 369 (6.3 ± 1.0)	0.8
SHBG, nmol/L	29.0 ± 3.9	26.5 ± 3.4	0.2
Insulin, μU/mL (pmol/L)	5.54 ± 0.82 (33.2 ± 4.9)	4.83 ± 0.87 (58.5 ± 5.2)	0.2
Glucose, mg/100 mL (mmol/L)	92 ± 2 (5.1 ± 0.1)	91 ± 3 (5.1 ± 0.2)	0.8
IGF-I, ng/mL or μg/L	305 ± 38	249 ± 32	0.02
IGFBP-3, mg/L	3.62 ± 0.19	3.4 ± 0.13	0.1
GH mean, ng/mL or μg/L	3.0 ± 0.7	2.0 ± 0.6	0.2
GH peak, ng/mL or μg/L	13.9 ± 2.9	11.9 ± 2.9	0.5
LH mean, mlu/mL or IU/L	2.5 ± 0.4	3.5 ± 0.4	0.0002
FSH mean, mlu/ml or IU/L	4.2 ± 1.4	5.8 ± 1.6	0.015
Osteocalcin, ng/mL	54.4 ± 11.5	55.8 ± 12.4	0.6
Bone alkaline phosphatase, U/L	36.2 ± 9.3	32.7 ± 9.9	0.1

GH, LH, and FSH were sampled every 10 min for 6 h. SI units are given in *parentheses*.
mlu, Milli-international unit.

TABLE 3. Whole body protein kinetics (micromoles per kg/min) as measured by [^{13}C]leucine kinetics and substrate oxidation rates (kilocalories per FFM/day) as measured by indirect calorimetry in healthy boys/young men treated with anastrozole for 10 weeks

	Day 1	Day 2	*P* value
Leucine Ra	1.87 ± 0.14	1.91 ± 0.09	0.8
Leucine oxidation	0.34 ± 0.03	0.31 ± 0.03	0.4
NOLD	1.53 ± 0.13	1.60 ± 0.08	0.6
Protein oxidation	6.2 ± 0.8	6.5 ± 1.0	0.6
Carbohydrate oxidation	9.5 ± 2.3	8.9 ± 2.1	0.7
Lipid oxidation	16.2 ± 1.8	11.6 ± 1.9	0.09

Leucine Ra represents whole body proteolysis; nonoxidative leucine disposal (NOLD) represents protein synthesis.

Protocol 2

Growth factors, substrates, and hormones. Table 2 summarizes the changes in hormones, growth factors, and glucose concentrations in the anastrozole-treated subjects. The 50% suppression of plasma E_2 observed in study 1 was sustained during the 10-week experiments, with a reciprocal increase in circulating testosterone. This was accompanied by a significant increase in circulating mean concentrations of gonadotropins, measured at frequent intervals for 6 h. There was no change in circulating sex hormone-binding globulin (SHBG) or DHEAS concentrations. IGF-I concentrations decreased; however mean and peak GH and IGFBP-3 concentrations remained invariant. Insulin and glucose concentrations did not change, and bone growth factors also remained invariant during the 10 weeks of anastrozole.

Protein kinetics and substrate oxidation rates. Rates of whole body proteolysis (leucine Ra), oxidation and synthesis [nonoxidative leucine disposal (NOLD)] remained invariant during estrogen suppression with anastrozole. Carbohydrate, lipid, and protein oxidation rates were also unaltered by treatment (Table 3).

Calcium kinetic analysis. Table 4 summarizes the measures of calcium absorption (α and Va) as well as bone calcium deposition (Vo^+) and bone calcium resorption (Vo^-) during these experiments; they all remained invariant. Urinary calcium excretion (Vu) did not change significantly during these studies.

Body composition and muscle strength. Total weight did not change during anastrozole treatment nor did measures of body composition (percentage of fat mass and fat-free mass) when measured by calipers, bioelectrical impedance analysis, and DEXA. Isokinetic and isometric measures of strength of the anterior quadriceps were not changed during estrogen suppression (Table 5).

Safety

All subjects had blood counts, urinalysis, and liver profiles performed routinely during these studies, and low and high density lipoprotein concentrations were measured. There were no changes in any of these parameters during the 10-week experiments and no reported side-effects. The drug was well tolerated.

Comparison with Lupron

These results contrast sharply with those reported by us in a similar group of young men (18–25 yr old) treated with the GnRHa Lupron and studied also 10 weeks apart (1, 28). In those subjects, GnRHa therapy significantly increased adiposity (percentage of fat mass; $P = 0.005$), decreased rates of

protein synthesis (NOLD; $P = 0.01$), decreased lipid oxidation ($P = 0.05$), decreased muscle strength ($P = 0.01$), and markedly increased urinary calcium losses ($P = 0.003$; Fig. 2). The E_2 suppression in those subjects was also more severe, *i.e.* 3.90 ± 0.61 pg/mL on day 1 *vs.* 0.25 ± 0.07 on day 2 (after Lupron).

Discussion

These studies have shown that specific blockade of the aromatase enzyme for 10 weeks did not have catabolic effects on protein metabolism or intermediate metabolism of substrates, and it did not affect body composition, quantifiable measures of muscle strength, or bone calcium metabolism in healthy eugonadal young men. These findings were observed despite a 50% reduction in circulating E_2 concentrations and contrast sharply with the profound catabolic effects observed after full suppression of the gonadal axis with GnRHa therapy in similar young men also studied 10 weeks apart, reported previously (1, 28).

TABLE 4. Calcium kinetic analysis before (D1) and after (D2) 10 weeks of daily anastrozole

	Day 1	Day 2	*P* value
α	0.297 ± 0.072	0.363 ± 0.058	0.20
Va	262 ± 82	332 ± 84	0.15
Vi	1032 ± 255	946 ± 227	0.15
Vu	137 ± 29	162 ± 19	0.21
Vo^+	1760 ± 370	1543 ± 237	0.16
Vo^-	1732 ± 378	1472 ± 247	0.14

α, Fractional calcium absorption; Va, total calcium absorption; Vi, dietary calcium intake; Vu, urinary calcium excretion; Vo^+, bone calcium deposition; Vo^-, bone calcium resorption. All units are milligrams per day, except α, which has no units.

TABLE 5. Changes in body composition, as measured by DEXA, and strength, as measured by isokinetic dynamometry of the knee extensors (anterior quadriceps), in boys/young men treated with 1 mg anastrozole for 10 weeks

	Day 1	Day 2	*P* value
BMI (kg/m^2)	21.2 ± 0.8	21.7 ± 0.8	1.0
Fat-free mass (kg)	55.0 ± 1.9	56.0 ± 1.9	0.3
% Fat mass (kg)	12.8 ± 1.9	12.9 ± 1.7	0.9
Extension peak torque (Nm, 60°/s)	160 ± 15	150 ± 15	0.2

To answer the first question of this study, the use of different doses of anastrozole (0.5 and 1 mg) was associated with comparable suppression of E_2 and reciprocal increases in testosterone and free testosterone concentrations without measurable changes in SHBG or IGF-I concentrations. We chose the 1-mg dose over the 0.5-mg dose because even though peripheral aromatase blockade appeared comparable to that with the 1-mg dose, it is possible that better aromatization blockade could be achieved at the tissue level with 1 mg. This in addition to the fact that the tablet is compounded as 1 mg made long term use of this drug easier. Even though this level of aromatase blockade achieved a 50% suppression of circulating E_2 concentrations, we cannot *a priori* decide whether this suppression is any less than that observed in male patients with the aromatase gene mutations. This is because the estrogen assay used in the present studies is substantially more sensitive than that used in the previously described patients. In the subject reported by Carani *et al.* (9), for example, the E_2 level was less than 10 pg/mL, whereas in our present study the assay sensitivity was 0.2 pg/mL; hence, whether this level of aromatase blockade is less than, the same as, or even more than that observed in patients with the mutations is unknown.

The biosynthesis of estrogens from C_{19} steroids is catalyzed by the aromatase cytochrome P450, a product of a single gene, *cyp19*. This enzyme is widely expressed in a multiplicity of tissues, including granulosa and luteal ovarian cells, Sertoli and Leydig cells of the testes, brain, adipocytes, liver, muscle, hair follicles, and, more recently, even bone (29–35). This enzyme converts androstenedione and testosterone to estrone and estradiol, respectively. After the administration of anastrozole, the 50% decrease in circulating E_2 and the corresponding increase in testosterone concentrations observed during the short term experiments to answer question 1 were preserved, indicating a persistent and efficient block of aromatization after 10 weeks of continuous exposure.

There were no changes in SHBG or adrenal androgen concentrations with this relatively long-term period of administration. Abundant data both *in vitro* and *in vivo* clearly show that E_2 administration is associated with an increase in circulating SHBG, whereas androgens decrease its concen-

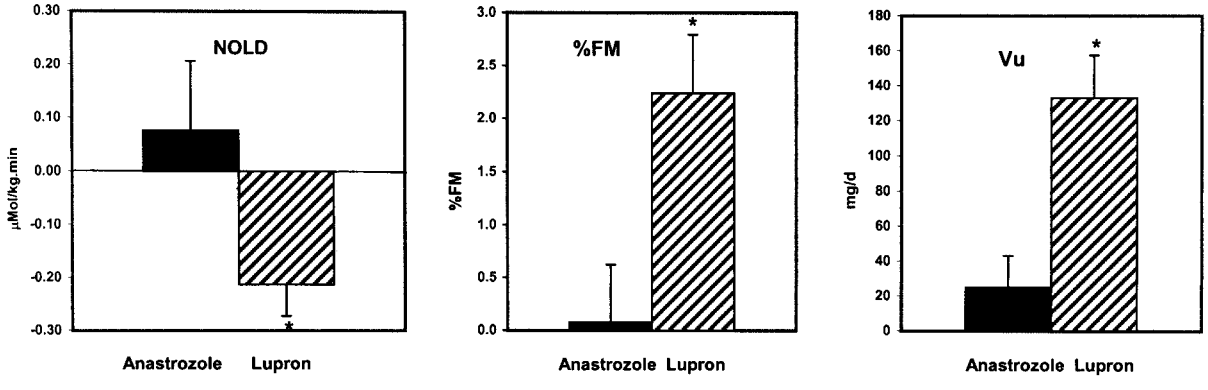

FIG. 2. Comparison of the changes observed after 10 weeks of anastrozole treatment (■; n = 8) *vs.* GnRHa therapy (▨; n = 6) in healthy boys and young men for whole body protein synthesis rates (NOLD), percentage of fat mass measured by DEXA, and urinary calcium excretion (Vu) measured by calcium tracers. Data are expressed as the absolute change from baseline. Comparisons were made using ANOVA between the groups.

tration (36). However, this regulatory system is more complex than reflected by the changes in SHBG concentrations. It is now known that SHBG binds to a cell membrane receptor, which causes the activation of cAMP, and this interaction only happens when sex hormones bind to SHBG (37). This ligand-receptor interaction was obviously not measured in the present paradigm, and it is not known what effects, if any, the changes in the sex steroid milieu had on this. As in the present model, E_2 concentrations decreased, with a reciprocal increase in testosterone; hence, the expected trend of a change in SHBG from the observed changes in sex steroid concentrations is negated. There was, however, a mild, but significant, increase in circulating gonadotropin levels. The latter supports the concept that E_2 is a major regulator of gonadotropin feedback, even in the male (8, 38, 39).

During the 10-day experiments there was a modest, yet significant, increase in DHEAS concentrations after using either 0.5 or 1 mg anastrozole; however, this increase was not observed after 10 weeks of aromatase blockade. These are interesting, yet puzzling, observations. DHEAS is almost entirely adrenal in origin and, in addition to DHEA and androstenedione, is the major androgen produced by the adrenal glands. Under normal physiological circumstances DHEAS seems to contribute little to circulating testosterone or E_2 concentrations, but could be converted to these steroids in certain tissues. It is conceivable that during the acute 10-day experiments DHEAS increased as a result of the aromatase blockade and the back-up of estrogen precursors. With more prolonged, persistent blockade of the tissue aromatase, it is possible that peripheral conversion of DHEAS contributed to the increase in testosterone concentrations. The latter consideration, however, is speculative and is not addressed directly in the present study.

Even though not apparent during the short, 10-day, dose-response experiments, prolonged administration of anastrozole resulted in a subtle, but significant, decline in IGF-I concentrations. This was, however, not associated with any detectable changes in circulating GH concentrations (as measured by frequent sampling) or plasma IGFBP-3 concentrations. This lack of effect on circulating GH concentrations was indeed somewhat unexpected, and it is possible that a larger number of subjects would have revealed a decrease in mean and peak GH concentrations, as six of eight subjects had a decrease in both of these measures. However, the relationship between estrogen and IGF-I may be complex and tissue specific, and other interpretations are plausible. Estrogen, for example, inhibits liver IGF-I production in hypophysectomized rats (40), and in postmenopausal women estrogen administration may attenuate the plasma IGF-I responses to exogenous GH (41), suggesting that in certain species estrogen may down-regulate hepatic GH receptors or impede tissue responses. It is possible that both androgens and estrogens participate in the regulation of GH production and that estrogen deficiency had an impact on the liver's output of IGF-I in the male. Regardless, it is important to carefully monitor plasma IGF-I concentrations in any subjects who are actively growing and are being treated solely with anastrozole.

Measurements of whole body anabolism and intermediate metabolism remained invariant during 10 weeks of aromatase blockade. Rates of protein synthesis and degradation, as measured by leucine tracer studies, and rates of carbohydrate, protein, and lipid oxidation did not change significantly during treatment. Body composition (weight, fat-free mass, percent fat mass) and skeletal muscle strength were not affected by the suppression of E_2 concentrations. These results differ significantly from the profound catabolic state observed in young males studied 10 weeks after GnRHa treatment with Lupron, in whom protein synthetic rates decreased, lipid oxidation rates decreased, adiposity increased, and muscle strength declined substantially after full suppression of the GnRH axis (1). These data suggest that suppression of estrogen concentrations at this level (50%) does not significantly affect large body protein pools and is congruent with observations in hypogonadal girls treated with estrogen reported previously (4).

The fact that we did not observe any detectable change in whole body protein kinetics or body composition despite the reciprocal increase in testosterone concentrations observed with anastrozole is also interesting. It is possible that this degree of increase in circulating androgens is not enough to see a change in protein kinetics and body composition with the metabolic tools of study used here. In the two models we used to show substantial anabolic effects of testosterone on whole body protein pools, the changes in testosterone concentrations were more profound than those observed here. In boys treated with testosterone (2) the testosterone concentration changed from 14 to 831 ng/dL, and in the young men rendered hypogonadal with Lupron (1) the decrease in testosterone concentrations was from 536 to 36 ng/dL, a 15- to 60-fold difference in concentrations, instead of the 1.5-fold difference observed here. Alternatively, the data could be interpreted to indicate that estrogen is necessary for the full anabolic effect of testosterone to be observed in the male. This requires further study.

The pivotal role of estrogens in the maintenance of skeletal mass is well characterized (42); hence, we were keenly interested in the effects of this level of suppression of E_2 concentrations in males on measures of calcium fluxes and bone health. Even though DEXA scans obtained before and after treatment did not show any changes in mineralization (data not shown), 10-week experiments are not long enough to detect changes in bone mineral density. However, serum markers of bone formation, osteocalcin, and bone-specific alkaline phosphatase remained invariant during these studies. In addition, calcium kinetic analysis using stable tracers of calcium showed no significant changes in urinary calcium excretion or kinetic measures of bone calcium accretion and resorption. These findings again contrast with the marked changes in calcium kinetics observed after GnRHa therapy in a comparable group of young men studied identically, in whom profound gonadal steroid suppression was associated with increased urinary calcium loses, increased bone calcium contribution to those loses, and increased bone calcium resorption (28). One potential explanation for these findings is that the length of these experiments (10 weeks) was not enough to detect changes in bone calcium metabolism; however, the later explanation is unlikely, as marked changes in these same parameters were observed after GnRHa therapy in subjects studied for 10 weeks (28). Alternatively, it is

possible that this degree of estrogen suppression at a 50% level may not have as profound an effect on bone as that observed after full estrogen receptor blockade (7) or complete gonadal steroid suppression (28). The reciprocal increase in testosterone concentrations may also serve a protective role of sorts in preventing bone loss, as androgens *per se* appear to have a critical effect as anabolic agents in bone (43).

Such a putative protective effect might not be operative, however, if the estrogen deficiency were complete and long term, such as that observed in patients with estrogen receptor and aromatase gene mutations (7–9). The fact that the few male subjects reported with either estrogen receptor or aromatase gene mutations all have had osteopenia clearly underscores the critical importance of estrogens in bone mineralization in the male; the data presented here do not contradict that. We can safely say, however, that within the narrow window of time of these studies (10 weeks), the same period of treatment with GnRHa in which we observed substantial negative effects in bone calcium metabolism in males of similar age (1, 28), we did not observe any deleterious effects in the exact same parameters as those used in those studies. Not only was the estrogen deficiency at the tissue level potentially more severe in the patients with aromatase and estrogen receptor mutations than that caused by anastrozole, but the length of the relative estrogen deficiency was longer by far in the patients than in the subjects reported here. It is hence prudent to carefully monitor bone mineralization and serum bone markers in subjects treated with any compounds that alter the sex steroidal milieu for a prolonged period. Continuous surveillance is clearly necessary.

GnRHa have been used extensively in short children in an attempt to delay epiphyseal fusion and increase ultimate height (44–46). Studies specifically looking at changes in body composition in this patient population are few, and the data are not consistent. Body mass index as the principal measure of adiposity was reported by Palmert *et al.* (47) in a group of 96 girls and 14 boys treated with GnRHa; it was found to be high in patients with precocious puberty even before treatment and was not worsened by therapy. This is a similar outcome as that observed by Heger *et al.* in 50 woman studied at final height (48). However, when better tools for the assessment of body composition (DEXA) are used prospectively and compared within patients with precocious puberty during treatment, fat mass and percent fat mass increased, whereas lean body mass decreased in a group of 34 children studied, of whom only 2 were boys (49). Our previously published data (1, 29) assess metabolic changes after GnRHa treatment and is perhaps the most extensive to date of any study; these data are congruent with those reported by Boot *et al.* regarding body composition. In addition to the lack of virilization caused by GnRHa, the detrimental effects on body composition; muscle strength; and protein, lipid, and calcium metabolism, make the use of these analogs unsuitable in the long term when the sole purpose of treatment is not precocious puberty *per se*, but to increase final height. The use of a potent selective aromatase blocker offers the advantage of continued virilization and maintenance of pubertal body composition while potentially delaying skeletal maturation. The latter is presently under investigation. Anastrozole treatment was well tolerated by all subjects. Glucose and insulin concentrations remained unchanged during these studies, as did plasma lipid concentrations, blood chemistries, and cell blood counts (data not shown).

In summary, this study of prolonged estrogen suppression for 10 weeks in young eugonadal subjects suggests that in the male estrogens do not contribute significantly to the changes in body composition and protein synthesis observed with changing androgen levels; estrogen is a main regulator of the gonadal-pituitary feedback for the gonadotropin axis; and this level of aromatase inhibition does not negatively impact markers of bone calcium metabolism, at least in the short term. In conclusion, 10 weeks of aromatase blockade in young males appears to be well tolerated and safe. Studies currently being conducted will provide valuable information on whether timed aromatase inhibition can be beneficial and safe in conditions of profound growth retardation in males. This strategy, if effective, may provide an advantage over the use of GnRHa to delay epiphyseal fusion.

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