

LETTERS TO THE EDITOR

Fractures after Long-Term Alendronate Therapy

To the editor:

In the September 2000 issue of your journal, a study by Tonino *et al.* (1) described the skeletal effects of 7 yr of treatment with alendronate. The study concluded that 7 yr of continuous treatment resulted in better skeletal effects than shorter therapy. I am concerned about the vertebral fracture rates that were presented in this paper. These fractures were confirmed by radiographs, but routine radiographs to check for vertebral fractures were not done. During the last 2 yr 3.3%/yr of women taking alendronate (10 mg/day) had a clinical vertebral fracture. This is higher than reported during the first 3 yr of this study, when the rate of morphometric vertebral fractures (defined by measurements from radiographs) was 2.1%/yr in the placebo group and 1.1%/yr in the alendronate groups (2). Direct statistical comparisons can't be made because there was no control group at yr 6 and 7.

The method of ascertaining fractures was different in the two papers. Other studies suggest that clinical vertebral represent only about a third of total vertebral fractures. For example, in the Fracture Intervention Trial (FIT), among women with a baseline vertebral fracture, the rate of new clinical vertebral fractures was 1.7%/yr in women taking placebo and 0.8%/yr in women taking alendronate. The rate of morphometric fractures was 5%/yr in the placebo group and 2.7%/yr in the alendronate group (3). In the other arm of the FIT study, where women did not have a fracture at baseline, the morphometric vertebral fracture rate was 0.9%/yr in the placebo group and 0.5%/yr in the alendronate group. The clinical vertebral fracture rate was only 0.2%/yr in placebo group and 0.1%/yr in the alendronate group (4).

In the women taking long-term alendronate, the rate of vertebral fractures was at least three times higher during yr 6 and 7 than during yr 1–3, despite the fact that the bone density of the spine was increasing. The rates were also higher than predicted from the data in the FIT study. The discrepancy cannot be explained by selection of more severe cases for the long-term study, because the baseline characteristics were similar. It also cannot be explained by aging of the population, because the FIT participants were older.

There is no doubt that alendronate increases bone strength and decreases fracture rate during the first 4 yr of use, but after that the profound suppression of the bone formation rate may begin to have a negative effect. I hope these findings will lead to further studies of the long-term effect of alendronate on bone strength.

Susan M. Ott
Division of Metabolism
University of Washington
Seattle, Washington 98195-6426

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Received November 27, 2000. Address correspondence to: Susan M. Ott, M.D., Division of Metabolism, Box 356426, University of Washington, Health Science Building, Room BB545, 1959 NE Pacific Street, Seattle, Washington 98195-6426.

Safety of Long-Term Alendronate

To the editor:

We agree with Dr. Ott that the long-term safety of alendronate is important because patients with osteoporosis may be treated for extended periods of time. However, we believe that the data support the use of alendronate through at least 7 yr. There are several reasons to be circumspect about Dr. Ott's comments.

Our study was too small to detect meaningful differences in fracture incidence in the 2-yr extension. At least 2000 women must be followed for 3 yr to have adequate statistical power for detecting a 40% reduction in fracture incidence (1). It is also not appropriate to attempt comparisons of fracture rates to other studies, or to the experiences of the same group at an earlier time, because incidence rates may vary between populations for reasons that are not apparent, even if characteristics such as bone mineral density seem to be similar, and it is well known that fracture rate increases with age (2). Although it was not mentioned in our paper, spinal radiographs were scheduled for all participants at the end of yr 7; new vertebral fractures (including asymptomatic fractures identified on radiographs) were identified by the local investigators and reported as adverse experiences. This difference in methodology, from the standardized morphometric assessment in yr 1–3, makes it impossible to perform valid comparisons of incidence rates and may explain, in part, why the incidence seems to be higher in yr 6 and 7. In fact, if one looks at vertebral fractures reported as adverse experiences by investigators during yr 1–3, the incidence of vertebral fractures in the patients who received alendronate was 3.8%/yr, which is quite comparable with the incidence of 3.3%/yr seen during yr 6 and 7. This is consistent with the similar rate of height decrease observed during yr 1–3 (1.0 mm/yr) and yr 6 and 7 (1.2 mm/yr).

We disagree with the hypothesis that "after (the first 4 years of use) the profound suppression of the bone formation rate may begin to have a negative effect." Both bone resorption and formation markers are substantially higher among postmenopausal osteoporotic women compared with premenopausal levels. As noted in our paper, bone turnover makers return to within the premenopausal range during treatment with alendronate and are not excessively suppressed. Treatment with alendronate (10 mg daily) produced a stable (~55%) decrease in bone-specific alkaline phosphatase, a marker of bone formation, such that mean bone-specific alkaline phosphatase was 8.3 U/L, well within the normal range for premenopausal women [mean (SD), 8.2 U/L (2.8); Ref. 3] after 7 yr of treatment. The continued increase in spine bone mineral density through 7 yr is further evidence that bone formation is not excessively suppressed.

Importantly, the annual incidence of nonvertebral fractures during yr 6 and 7 was not substantially different than during yr 1–3; this does not support any increase in fracture incidence with longer use of alendronate. In conclusion, we do not believe there is any evidence of an increase in fracture risk with continued use of alendronate up to 7 yr.

Richard P. Tonino, Arthur Santora, and Philip D. Ross
Good Health, P.C. (R.P.T.), South Burlington, Vermont 05403;
and Merck and Company, Inc. (A.S., P.D.R.), Rahway, New Jersey

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Received January 22, 2001. Address correspondence to: Richard P. Tonino, M.D., 368 Dorset Street, Suite 1, South Burlington, Vermont 05403.

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Aromatase Inhibitors in Pubertal Boys: Clinical Implications

To the editor:

I enjoyed the manuscript by Mauras *et al.* (1) because it opens interesting perspectives in the possible clinical use of aromatase inhibitors (AIs) in the treatment of growth disorders, as previously suggested by Grumbach and Auchus (2). AI constitutes a selective tool useful to obtain a delay in epiphyseal closure during skeletal development. The advantage of using AIs consists in a selective block of estrogen action without affecting biological effects due to androgens *per se* (1). Thus, a pharmacological “decoupling” of androgen action from that of its estrogen metabolite is reached. In this view, AIs permit to extend longer the time of growth in boys with growth disorders (*e.g.* GH deficiency, idiopathic short stature), thus leading to an increased powerful of GH therapy and to a better final height. Presently, skeletal maturation could be restrained only by using GnRH analogs. AIs, however, do not affect the progression of pubertal development because a block of estrogen action could occur together with a normal advancement of virilization. Thus, AIs could prevent the psychological problems related to delayed puberty that often occur when GnRH analogs are used.

The authors state that AIs are safe in young adolescents (1), and this is the case if a short-term period of treatment is considered. By contrast, the issue becomes very complex if the treatment is carried out for long time, as it is necessary in the case of short stature, being a great prolongation of the period available for growth desirable to fill the gap of height. Several issues need to be clarified before AIs will be considered safe at puberty. Puberty, in fact, is the time for the children to mature sexual organs, to reach both adult proportions of the skeleton and a complete adult psychosexual behavior. If undesirable effects due to pharmacological treatments occurred in this fragile phase of development some physiological functions could be compromised forever.

Received October 31, 2000. Address correspondence and requests for reprints to: Vincenzo Rochira, M.D., Cattedra di Endocrinologia, Università di Modena e Reggio Emilia, Policlinico di Modena, Via del Pozzo, 71, 41100 Modena, Italy. E-mail: rochira.vincenzo@unimo.it.

Before analyzing the hypothetical disadvantages of AIs, estrogen actions at puberty in the male have to be underlined. First, estrogens act on bone tissue in men. Epiphyseal closure and skeletal maturation do not happen without estrogens (2, 3). The finding of eunuchoid proportions of the skeleton in estrogen-deficient men (2) suggests that estrogens are involved in the establishment of the proportions of the growing skeleton, leading to eunuchoid body habitus (3). It seems that growth spurt also is an estrogen-dependent process (2), although a conclusive consent on this issue has not been reached, because a detailed growth chart of estrogen-deficient men has never been provided (2, 3). Estrogens are needed to achieve the peak of bone mass (2, 3) during early to late adolescence.

Second, estrogens could modulate reproductive function in men. Aromatization of androgens is required for a normal male sexual behavior in mice, but much less is known about estrogens and human male sexuality (4). Estrogens are necessary for a normal fertility in male rodents. Presently, more research is required to prove whether the same mechanisms operate in the human testis, although recent findings (*e.g.* the presence of estrogen receptors and aromatase activity in the male reproductive system) strongly support an estrogen role on human spermatogenesis. In any case, estradiol is the major modulator of gonadotropin secretion in normal men, acting both at pituitary (1, 2, 3, 5) and hypothalamic levels (6), and estrogens are consequently involved in the control of testicular function.

Third, abnormalities of lipid and carbohydrate metabolism in men with congenital estrogen deficiency (2, 3) suggest that estrogens could modulate some metabolic pathways, having a protective role also on the male cardiovascular system (2).

Stated both well-documented and supposed effects of estrogens in men, it should be considered that AIs could interfere with each of these physiological processes at puberty (Table 1). A delay in skeletal maturation could induce a disproportional growth, accounting for final eunuchoid body proportions. A peak of bone mass lower than normal could result from AI administration, predisposing the occurrence of osteoporosis later during adulthood. Accordingly, spermatogenesis, the psychosexual behavior, the cardiovascular system, and some metabolic pathways also could be impaired with an increased risk for health during adulthood.

AIs directly decrease serum estradiol with an increase of the testosterone to estradiol ratio (1). The imbalance between circulating sex steroids is more severe if we consider also the effects of AIs on gonadotropin feedback (6): higher LH levels cause a testosterone overproduction without a concomitant increase of estradiol (1), a

TABLE 1. Role of estrogen at puberty in the male and possible advantages and disadvantages of using AIs in young adolescent males

Estrogen actions at puberty in the male	Advantages of using AIs	Disadvantages of using AIs
Skeletal development		
Epiphyseal closure and growth arrest ^a	Slowing down epiphyseal maturation delays growth arrest and prolongates growth. Potential benefits in growth disorders alone or in combination with r-hGH therapy.	A delay in epiphyseal closure and growth arrest could lead to disproportional growth of some skeletal districts: eunuchoid body proportions.
Achievement of peak bone mass ^a	None	Decreased peak of bone mass. Increased risk of osteoporosis during adulthood.
Growth spurt ^b	Absence of growth spurt? Continuing linear growth.	Reduced height velocity? Eunuchoid body proportions.
Establishment of final skeletal proportions ^a	None	Eunuchoid body proportions.
Feedback of gonadotrophins ^a	Decreased circulating estrogens	Increased serum LH and Testosterone. Severe imbalance of testosterone to estradiol ratio. Macroorchidism?
Fertility ^b	None	Risk of disrupted spermatogenesis. Macroorchidism?
Behavioral changes ^b	Continuing skeletal growth together with an unaffected virilization with a positive psychological effect (especially when compared with GnRH analogs).	Risk of an impairment of both the development and the achievement of a normal adult male psychosexual behavior.
Cardiovascular system and metabolic pathways ^b	Uncertain	Uncertain

^a Certain.

^b Uncertain.

hormonal pattern accounting for macro-orchidism in congenital estrogen-deficient men. What are the consequences due to such a severe imbalance in circulating sex steroids during an important phase of development, which is puberty? Awaiting an answer to this question from future studies, some ethical perspectives should be considered. The aim of this letter is to consider clinical implications related to the pioneering use of these drugs in boys to promote a successful debate among researchers on the possible inherent risks and benefits. The real impact of AIs on several physiological processes, which take place at puberty, remains to be well established because a well-conducted, controlled clinical trial has never been performed. Presently, although the unique work performed by Mauras *et al.* (1) opens this novel form of therapy to study, it also leaves several questions unanswered. In that short-term treatment, the effects of AIs on skeletal maturation, fertility, and sexual behavior were not studied in detail (1). Besides, caution is needed when data are extrapolated from a short-term treatment because a drug affecting physiological processes related to the developing body reveals its potential negative effects when a long-term treatment is performed. What's more, the issue is complicated by the fact that some adverse events could be detectable only later during adult life (*e.g.* infertility).

Again, what is the impact of AIs on local estrogen production? A block of estrogenic functions involves both autocrine and paracrine mechanisms in each tissue in which aromatase is expressed. One of the tissue-specific implications related to the iatrogenic "decoupling" of androgen from estrogen action in adolescent boys is represented by environmental changes in the testis (testosterone is higher with estradiol lower than normal). Are the consequences on spermatogenesis occurring later during adulthood predictable? The answer to this latter question is not so easy. Presently, in fact, we still do not know in detail the effects on male fertility of the treatment with GnRH analogs during adolescence (7).

To establish both the true rates of possible adverse events and the efficacy of AIs in male growth disorders, results from a long-term treatment are anxiously awaited. Obviously, the following parameters should be carefully monitored: sperm count, testicular size and morphology, height velocity, anthropometric measurements of several skeletal districts, bone mineral density, and psychosexual behavior. Changes in these parameters during long-term treatment of adolescent boys will permit to establish in detail the side effects of these drugs. Presently, it is only possible to speculate on some adverse events only by starting from our knowledge on the physiological estrogen role in males. Thus, also progress in the field of estrogen physiology will probably permit us to forecast side effects due to the experimental use of AIs in boys.

In conclusion, AI has to be regarded as an experimental regimen at puberty, being a therapeutic approach not miming physiological pubertal changes of sex steroids. To transfer the use of AIs in pubertal boys into clinical practice, results from clinical trials on long-term treatment need to be available and researchers need to clarify all of the androgen actions that are really to be ascribed to estrogen. Accordingly, until much larger studies on these two issues have been done, physicians should continue to exercise caution with regard to the potential for adverse effects of AI administration to male adolescents.

Vincenzo Rochira
Department of Internal Medicine
University of Modena and Reggio Emilia
41100 Modena, Italy

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Author's Response: Aromatase Inhibitors in Pubertal Boys—Clinical Implications

To the editor:

I appreciate the opportunity to reply to the letter of Dr. Rochira regarding the use of aromatase inhibitors in pubertal boys.

In our work published in *JCEM* this summer (1), we report our results on the investigation of the intermediate metabolism of substrates, bone calcium metabolism, body composition and strength in young males given an aromatase inhibitor (Anastrozole) for 10 weeks and compared the results with those of identical experiments in young men given a GnRH analog. In it, we clearly restrict our conclusions to the effects observed over 10 weeks and only speculate to the potential use of this compound in delaying epiphyseal fusion in short pubertal boys. I personally agree with the substance of the concerns expressed by Dr. Rochira, and I echo the warning that the use of this and other similar aromatase inhibitors cannot be advocated until careful, methodical studies, like the one reported, can be carried out in a large enough group of patients and the data analyzed. Much more work needs to be done, and such studies are presently underway.

That estrogen is important in bone health is not questioned here. Actually, we used the model of aromatase inhibition with concomitant GH treatment in pubertal boys, and preliminary results show no detrimental effects on bone mineralization after 1 yr of combined treatment (unpublished observations). More data are also needed on the issue of the impact of estrogen deficiency on sperm function and fertility. More is written about the negative effects of estrogen exposure to the testis than the effects of estrogen deficiency. Animal data clearly offer mixed results with both apparent disruption of spermatogenesis in mice lacking functional aromatase (2), and no difference in the number of Sertoli cells, germ cells, the volume of seminiferous epithelium, tubule lumens, and interstitium between controls, and Anastrozole-treated rats after 1 yr (3). Extrapolating the effect of complete, lifelong aromatase blockade on sperm function from the published report of the man with this aromatase deficiency (whose brother also had decreased sperm counts even though he had no aromatase gene mutation; Ref. 4), to the effects of timed pharmacological aromatase blockade may not be directly comparable. The latter achieves a 50% reduction in circulating estrogen concentrations and not a complete suppression. A critical question yet to be answered, however, is not only if this intervention, like treatment with a GnRH analog, affects sperm function, but more importantly, is it reversible after discontinuation of treatment. This family of compounds, and the new class of estrogen receptor blockers, will require thorough and analytical study in the years to come. Only when proven efficacious and safe in long-term studies, can their potential usefulness in the pharmacological arsenal available to the clinician be fully assessed.

Nelly Mauras
Division of Endocrinology
Nemours Children's Clinic
Jacksonville, Florida 32207

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Received December 4, 2000. Address correspondence to: Nelly Mauras, M.D., Division of Endocrinology, Nemours Children's Clinic, 807 Nira Street, Jacksonville, Florida 32207.

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Practical Classification of Prolactinomas for Clinical Use

To the editor:

Notable discrepancies appear when analyzing the results from various studies assessing treatment outcomes of patients with prolactinomas. Recently, based on a retrospective review of 46 male patients with hyperprolactinemia managed with dopamine agonist (DA) therapy alone, Pinzone *et al.* (1) conclude that PRL normalization is comparable in male patients with macroprolactinomas *vs.* those with microprolactinomas. This study also shows that the response rates to bromocriptine (BRC) and cabergoline (CAB) were similar in both groups. This is rather surprising because CAB has been previously reported to be more effective than BRC in normalizing PRL levels in patients with prolactinomas (2) and because macroprolactinomas in men have been shown to be more aggressive tumors with a higher prevalence rate of invasion toward cavernous sinus and resistance to BRC (3). Moreover, this is not in keeping with the results from the large-scale study by Verhelst *et al.* (2), who found that the probability of reaching normal PRL levels during CAB treatment was significantly higher in patients of both sexes with a microprolactinoma than in patients with a macroprolactinoma. Thus, what is wrong? Probably the absence of uniform classification of patients with presumed prolactinomas.

In the study by Pinzone *et al.* (1), inclusion criteria for "microprolactinomas" ($n = 12$) were a serum PRL level more than 15 ng/mL and either a normal computed tomography or magnetic resonance imaging scan of the pituitary or a tumor less than 1 cm in diameter. Obviously, this definition includes patients with idiopathic hyperprolactinemia. Although sexual dysfunction was present in all cases, testosterone levels were normal in 3 of 11 patients. In this setting, it has been recommended to rule out the presence of a biologically inactive macroprolactinemia (4). On the other hand, 4 of 11 patients remained testosterone deficient at follow-up despite normalization of PRL levels in at least 2 of them. The absence of restoration of gonadal function in men with small prolactinoma and mild testosterone deficiency is unusual (5), which suggests that PRL was not the cause of hypogonadism and should prompt the search for alternative diagnosis. Finally, it must be remembered that the finding of a "microadenoma" on scan in a patient with moderately elevated PRL levels may not always indicate the presence of a prolactinoma (6). Thus, the small group studied probably represents a highly heterogeneous population. Nevertheless, normalization of PRL levels (<15 ng/mL) was achieved in 83% (10 of 12 patients). Moreover, it must be stressed that the two patients considered "DA resistant" had also nearly normal PRL values (between 15 and 17.5 ng/mL). Regarding macroprolactinomas, the study did not consider patients with tumors invading the cavernous sinuses separately, although a preliminary report indicates that invasive macroprolactinomas may be more frequently resistant to BRC than noninvasive ones (3). Highly aggressive macroprolactinomas often require multiple treatment modalities, but the authors excluded patients treated by surgery and/or adjunctive radiotherapy. Thus, the more aggressive macroprolactinomas should logically be searched for in the large group of patients ($n = 77$) excluded from the study.

In conclusion, the study by Pinzone *et al.* (1) shows that PRL normalization is comparable in a group of male patients with idiopathic hyperprolactinemia or presumed microprolactinoma and in a selected group of patients with macroprolactinomas not requiring surgery or radiation therapy. To facilitate comparison of treatment results between centers, additional studies should refer to a precise classification of prolactinomas. We propose such a classification in Table 1, which mainly relies on the following evidences: microprolactinomas have a benign course and rarely progress in size over time; invasion of the cavernous sinuses can be determined by radiological criteria and is associated to higher proliferation activity; DA-resistant prolactinomas have been

TABLE 1. Classification of prolactinomas according to an ascending degree of aggressive behavior

1. Microprolactinoma (pituitary tumor of <10 mm in diameter associated with symptomatic hyperprolactinemia)
2. Macroprolactinoma (pituitary tumor of at least 10 mm in diameter associated with PRL levels usually over 200 ng/mL)
3. Invasive prolactinoma (restricted to invasion of the cavernous sinuses space)
4. Aggressive prolactinoma (defined as invasive and DA resistant)
5. Metastatic prolactinoma

shown to exhibit a more severe clinical course both in human and in animal models; and combined invasiveness and DA resistance usually does not allow normalization of PRL levels, whatever the choice of therapy. The proposed classification may also help the clinician to plan the treatment and inform about the prognosis.

Etienne Delgrange and Julian E. Donckier
Department of Internal Medicine and Endocrinology
Université Catholique de Louvain at Mont-Godinne University
Hospital
B-5530 Yvoir, Belgium

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Medical Therapy of Prolactinomas in Men

To the editor:

Drs. Delgrange and Donckier have raised several issues concerning our study regarding primary dopamine agonist treatment in men with prolactinomas. As the authors correctly state, our study shows that the rate of PRL normalization in response to dopamine agonists is comparable in men with micro- or macroprolactinomas. These male subjects had not undergone surgery or radiation therapy and, thus, were treated with dopamine agonists alone. The men presented with a spectrum of radiographic and clinical findings, and the heterogeneity of prolactinoma presentation is well established. The diagnosis of "idiopathic hyperprolactinemia" is somewhat problematic because such patients are often found to have lesions either at surgery or on magnetic resonance imaging scans as the sensitivity of such scans improves over time. As Drs. Delgrange and Donckier suggest, invasive macroprolactinomas may respond differently to dopamine agonists than noninvasive ones. However, the intent of the study was to present the results of men with prolactinomas treated with medical management alone without preselecting a defined group. Prolactinoma responsiveness to therapy is often difficult to judge based on proven biologic, endocrinologic, or pathologic classification. Invasive prolactinomas may respond to dopamine agonist therapy with marked decreases in tumor size and normalization of PRL levels. In contrast, patients with microprolactinomas may show resistance to dopamine agonist administration presumable due to differences

Received November 20, 2000. Address correspondence to: Etienne Delgrange, M.D., Department of Internal Medicine and Endocrinology, Université Catholique de Louvain at Mont-Godinne University Hospital, Solidarité Mutualiste Chrétienne, B-5530 Yvoir, Belgium.

Received December 1, 2000. Address correspondence to: Anne Klibanski, M.D., Neuroendocrine Unit, Massachusetts General Hospital, 55 Fruit Street, Bulfinch 457B, 5-194 Joseph, Boston, Massachusetts 02114.

in dopamine receptor concentration or binding characteristics. Therefore, we categorized patients based on magnetic resonance imaging scan size.

Drs. Delgrange and Donckier question our finding of similar efficacy between dopamine agonists. The only way to determine whether one dopamine agonist is more effective than another is through a prospective, randomized, controlled study of both agents. Given the inherent limitations of a retrospective chart review, one can only make conclusions on PRL normalization in treated patients. Clearly, prospective studies are warranted to address this specific question.

Finally, the natural history and therapeutic response to dopamine agonists of microprolactinomas has been well characterized in previous prospective as well as retrospective series in women. However, the natural history of untreated microprolactinomas in men has not been well characterized due to the necessity of treating the low testosterone levels or sexual dysfunction that typically leads to presentation. Furthermore, there are few data regarding the therapeutic responses to dopamine agonist therapy in men. Certainly a better understanding of this disease in men would be of benefit to this increasing population of patients with this diagnosis.

Anne Klibanski and Laurence Katznelson
Neuroendocrine Unit
Massachusetts General Hospital
Boston, Massachusetts 02114

Physical Performance in Growth Hormone-Deficient Adults

To the editor:

We have read with interest the paper by Woodhouse *et al.* (1), dealing with the evaluation of submaximal aerobic performance in adults with adult-onset GH deficiency (GHD) before and after recombinant GH (rec-GH) treatment.

The authors point out how the perception of increased fatigue and impaired physical performance experienced by these patients derives from a markedly reduced maximal aerobic capacity ($\dot{V}O_{2\max}$). Thus, even the execution of ordinary daily activities requires a greater fraction of $\dot{V}O_{2\max}$, imposing to the patients a discomfort out of proportion to their exercise task.

The study by Woodhouse *et al.* (1) suggests that these changes may be, in part, related to the lack of GH-insulin-like growth factor I actions on muscle mass, because the rec-GH treatment was associated with a marked increase in skeletal muscle insulin-like growth factor I messenger RNA, improving aerobic capacity indices and self-reported fatigue during low-intensity exercise.

A picture thus emerges, in line with a common view (2, 3), of the adult-onset GH-deficient patient as a poor performer, provided with a below normal amount of contractile elements devoted to locomotor tasks and to cardiorespiratory support of exercise.

Our group reached quite different conclusions in studies of short-statured childhood-onset GH-deficient adults, which seem to have a normally "proportioned" contracting machinery (4–6). In fact, although absolute values of quadriceps strength and fiber cross-sectional area (CSA) of patients were significantly lower than controls (4), differences disappeared once the absolute values were normalized for quadriceps CSA and the subjects' height. Normal muscle twitch kinetics and fatigability in the quadriceps of GH-deficient patients were fully consistent with the lack of a significant shift in fiber type proportion (4).

In another recent study from our group (5), analyzing the anaerobic performance of adults with GH deficiency, we concluded that both lactacid and alactacid maximum anaerobic power were similar in controls and patients when absolute differences (35%) were adequately normalized for body mass. These findings indicate that the GH-deficient patients' ability to sustain anaerobic power and their rate of fatigue were comparable, in relative terms, with those of healthy controls.

Furthermore, as far as the aspects of mechanics and energetics of

locomotion (walking and running) are concerned (6), we have clearly demonstrated that patients and healthy controls moved with the same metabolic cost and efficiency of locomotion, provided that walking and running velocities are expressed as Froude number (which takes into account the scale differences between the two groups). Nevertheless, it is noteworthy that our patients with childhood-onset GHD were actually unable to run at speeds higher than 8 km/h^{-1} for the time needed to reach a metabolic steady state (6). At this maximally attained speed their specific $\dot{V}O_2$ was about $25 \text{ mL/min}^{-1} \cdot \text{kg}^{-1}$ and their average heart rate was about $180 \text{ beats/min}^{-1}$, which, from their measured resting and age-estimated maximum heart rate, would correspond to about 90% of the maximum, strongly suggesting a remarkable reduction in $\dot{V}O_2 \max$ also in childhood-onset patients with GHD.

The considerable differences between patients with childhood-onset and adult-onset GHD (related to the duration, age of appearance, degree of the disease) make mandatory for the future to analyze separately the data recorded in so different clinical conditions.

Moreover, the apparent discrepancy between the effects of rec-GH on muscle strength (which remained unchanged) and submaximal aerobic performance (significantly improved), as reported by Woodhouse *et al.* (1), warrant further attention and should be the focus of future studies investigating the effects of rec-GH on muscle.

Alessandro Sartorio, Claudio Lafortuna, and Marco V. Narici
Istituto Auxologico Italiano (A.S.), IRCCS,
20145 Milano, Italy; Istituto Tecnologie Biomediche
Avanzate (C.L.), CNR, 20090 Milano, Italy; and
Department of Exercise and Sport Science (M.V.N.),
Metropolitan University of Manchester, Alsager, United Kingdom
ST7 2HL

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Growth Hormone Deficiency and Physical Function

To the editor:

We agree with Dr. Sartorio and his colleagues that the effects of rhGH treatment on muscle size, strength, and anaerobic capacity in adult GH-deficient (GHD) patients are limited. We did not see marked improvement in muscle strength despite an increase in mean fiber area (both type I and type II fibers) following rhGH treatment as compared with placebo or baseline (1). However, the significant improvement in aerobic function that we have described (1) suggests that the capacity of skeletal muscle to utilize oxygen for energy expenditure maybe enhanced and/or there is an enhanced ability to deliver oxygen in response to rhGH treatment. The improvement in cardiac function associated with rhGH treatment (2) is certainly compatible with the latter possibility. Nevertheless, it may well be that the interface of oxygen delivery

Received December 2, 2000. Address correspondence to: Shereen Ezzat, M.D., Division of Endocrinology, Mount Sinai Hospital, University of Toronto, 600 University Avenue, Suite 429, Toronto, Ontario, Canada M5G 1X5.

Received March 2, 2000. Address correspondence to: Alessandro Sartorio, M.D., Research Center for Growth Disorders, Italian Institute for Auxology, Via Ariosto 13, 20145 Milan, Italy. E-mail: sartorio@auxologico.it.

and utilization is critical in GHD subjects and that examination of muscle function alone may be less revealing in the GH deficiency state.

Dr. Sartario's studies comparing childhood-onset GHD adults to age-, sex-, and activity-matched control subjects suggest that the reduced muscle size and strength in GHD adults is a function of reduced body size. Although this explanation is plausible for short-statured adults who have been GHD since childhood, this does not seem to be the situation for GHD adults who have a normally developed body size. Moreover, while correction for reduced body size in adults with childhood-onset GHD may negate the physiological differences seen in absolute terms, the functional consequences cannot be so easily dismissed. The fact remains that to perform activities of daily living (such as walking, climbing stairs, rising from a chair, *etc.*) these GHD adults must move a larger body mass, carrying a heavier load of fat with less metabolic machinery available to perform the work than their non-GHD counterparts.

We agree with Dr. Sartario and his colleagues, who suggest that future studies need to consider the onset of GHD. Such differences may be a potential source contributing to the variable responses to GH treatment on muscle and functional performance in GHD adults.

Linda J. Woodhouse, Sylvia L. Asa, Scott G. Thomas, and
Shereen Ezzat
University of Toronto
Toronto, Ontario, Canada M5G 1X5

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Optimal Range of Plasma Concentration of True 1–84 Parathyroid Hormone in Patients on Maintenance Dialysis

To the editor:

We have read with interest the rapid communication of John *et al.* (1) showing that the new Scandibodies laboratory immunoradiometric assay (IRMA) for measurement of intact PTH detects full-length human PTH but not amino-terminally truncated fragments, in contrast to most commercially available IRMAs for so-called intact PTH, which measure also 7–84 PTH as shown by Lepage *et al.* (2).

In contrast to the Nichols Institute Allegro IRMA for intact PTH (N-IRMA), our PTH IRMA (B-IRMA) (Ref. 3; later commercialized by Biosource for the last 10 yr) had the particularity to have a much lower normal range of 3–40 pg/mL *vs.* 10–65 pg/mL for N-IRMA. The reason for this discrepancy was not clear until 1992 when we compared the recovery of 7–84 PTH fragments at a wide concentration range in our assay and in that of Nichols Institute. The recovery of fragment (7–84) was 100% with N-IRMA and less than 1% in B-IRMA. Therefore, the nondetection of 7–84 was a satisfactory explanation for the lower normal range.

The clinical relevance of such difference is quite obvious for the indirect evaluation of bone remodeling in hemodialysis patients. In 1991, Cohen Solal *et al.* (4) published in this journal the (1–84) PTH levels with the B-IRMA of 23 hemodialysis patients for whom a bone biopsy had been performed. This allowed us to classify six of them in the adynamic bone disease (ABD) group [low bone formation rate (BFR) with low osteoid thickness and low osteoblastic and osteoclastic surfaces], eight in the mild lesion group (with normal BFR and mild increase in osteoblastic and osteoclastic surface), and nine in the osteitis fibrosa group (with high BFR and increased osteoblastic and osteoclastic surfaces). All the nine patients with osteitis fibrosa had PTH levels greater than or equal to 69 pg/mL (*i.e.* 1.7 the upper limit of normal). On the contrary, all the six patients with ABD had PTH levels within the normal range. Four of eight patients with mild lesion had PTH levels within the normal range, three being above and one below. Considering that an intact PTH

range associated with normal BFR could define its optimal level in hemodialysis patients, we proposed that their Biosource B-IRMA PTH should be between 40 and 60 pg/mL (*i.e.* between 1 and 1.5 the upper limit of normal to prevent adynamic bone disease). This proposed optimal range is quite lower than that proposed with N-IRMA not only when expressed in absolute concentrations but also when expressed in folds of the upper limit of normal (ULN) since Quarles *et al.* (5) in 1992 and Wang *et al.* (6) in 1995 proposed in hemodialysis patients, 1.5–2.5 and 2–4 times the ULN, respectively, the upper threshold being a little higher in CAPD patients according to Wang *et al.* (Ref. 6; 5 times the ULN).

Four hypotheses may be discussed to explain the discrepancy between our proposition for optimal PTH range and those of our American colleagues: 1) the role of a possible 7–84 PTH fragment accumulation in renal failure; 2) the role of the antagonistic effect of 7–84 PTH fragments on the bone remodeling effect of endogenous 1–84 PTH; 3) the role of a difference in aluminum overload leading to a higher bone resistance to endogenous PTH remodeling effect; and 4) the role of calcitriol treatment.

1. Accumulation of 7–84 in renal failure is a possibility to consider since Brossard *et al.* (7) showed with high-performance liquid-chromatography determination that the proportion of non-1–84 PTH fragments in the intact PTH detected by N-IRMA was greater in hemodialyzed patients than in healthy controls whether the patients are in stable condition (50 *vs.* 21%), have PTH stimulation by hypocalcemia (36 *vs.* 13%), or PTH suppression by hypercalcemia (65 *vs.* 25%). However, in the article by John *et al.* (1) the proportion of non-1–84 PTH recognized by N-IRMA is not much greater in dialysis patients than in healthy controls. Indeed, we calculated it by using the ratio of the ULN range to be 52% in controls (65–31/65) and 59% in the eight dialysis patients (688–277/688) in basal condition and 50% after stimulation by citrate-induced hypocalcemia. Only when PTH secretion was suppressed by calcium infusion was the proportion much higher (78%), this latter phenomenon being easily explained by the well known increased catabolism of intact 1–84 PTH to C-terminal fragments within the parathyroid glands (8). Furthermore, Slatopolsky *et al.* (9) recently showed that the proportion of non-1–84 PTH fragment recognized with N-IRMA was not lower in transplanted patients with better renal function than in dialysis patients since the contrary is rather observed: 44% *vs.* 34%. Thus, the 1.5- to 5-fold higher PTH levels found necessary to maintain normal BFR in the dialysis populations of Quarles *et al.* (5) and Wang *et al.* (6) cannot be totally explained by a disproportionate retention or secretion of non-1–84 fragments, since the proportion of these latter has been documented only marginally higher in stable dialysis patients than in nonuremic patients in the study of John *et al.* (Ref. 1; 59% *vs.* 52%).

2. The hypothesis of Slatopolsky *et al.* (9) that the 7–84 PTH fragments could, furthermore, have an antagonistic action *vs.* endogenous 1–84 PTH on bone remodeling in uremic patients is, therefore, quite attractive. This hypothesis is based on the observation that human 7–84 PTH (hPTH 7–84) did not increase cAMP production in osteoblast-like cells and antagonized in nonuremic rats the hypercalcemic and phosphaturic effects of hPTH 1–84. However, to support the conclusion that this antagonistic effect proven in nonuremic rats is the explanation for the development of ABD in dialysis patients, one would like to document a higher proportion of non-1–84 PTH measured by N-IRMA in patients with ABD than in those with osteitis fibrosa. Since according to John *et al.* (1) this proportion is rather lower (52% and 55%) in the two patients with ABD than in the six patients with osteitis fibrosa (61% and 59%), we do not think that this explanation is quite satisfactory.

3. Therefore, we think that the main reason for the discrepancy between our proposition regarding the optimal range for intact PTH is related to a greater bone resistance to the PTH remodeling effect in the American dialysis patients than in the Amiens dialysis patients, in relation to their higher exposition to aluminum. Indeed, none of the Amiens patients had traces of aluminum staining on their bone biopsy because they had never been exposed to aluminum either by the dialysate or by the phosphate-binders, these latter having been definitively excluded since 1980. On the contrary, even though Quarles *et al.* (5) excluded patients with stainable aluminum, aluminum phosphate-binders were still prescribed in their center. As regards the patients of Wang *et al.* (6), only those with more than 25% of their osteoid/calcified bone interfaces positive for aluminum were eliminated, so that most of them had positive aluminum staining from 1–24% of their bone interfaces because of previous aluminum-phosphate-binder ingestion. Since it is well documented that aluminum has a direct inhibitory effect on os-

Received October 9, 2000. Address correspondence to: Dr. A. Fournier, Hôpital sud Service de Néphrologie Médecine Interne du CHU d'Amiens, Avenue René Laënnec, 80054 Amiens, France.

teoblasts, even when no stainable aluminum is present (10), it is very likely that the difference in aluminum overload is the main explanation for the differences in optimal range of PTH between our center and the American ones.

4. The role of treatment by calcitriol has to be eliminated since calcitriol treatment even without aluminum phosphate binder can further down-regulate PTH-PTHrP receptor (11) and decrease high bone remodeling without a significant decrease of plasma intact PTH (12, 13). This factor can, however, be eliminated as an explanation for our discrepancy with the proposal of Wang *et al.* (6) since none of our patients and of those of Wang *et al.* (6) were receiving calcitriol.

The fact that in our unique population of patients without exposition to aluminum, or calcitriol, we documented a subpopulation with truly normal secretion of PTH and low BFR, formally establishes for the first time that the uremic bone of these patients is not only resistant to the hypercalcemic effect of exogenous PTH [shown by Massry *et al.* (14) in 1974] but also to the remodeling effect of endogenous PTH.

A. Fournier, M. E. Cohen Solal, R. Oprisiu, H. Mazouz, P. Morinire, G. Choukroun, and R. Bouillon
Nephrologie—CHU (A.F., M.E.C.S., R.O., H.M., P.M., G.C.),
Amiens, France; and LEGENDO University Hospital (R.B.)
Leuven, Belgium

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Parathyroid Hormone-Immunoradiometric Assays as Noninvasive Predictors of Renal Osteodystrophy: The Need for Bone Histomorphometric Validation

To the editor:

The comments of Fournier *et al.* emphasize the importance of adequately documenting the relationship between serum or plasma PTH

concentrations as determined by any particular assay method and the histological features of bone in patients with renal osteodystrophy. Such information is essential to properly apply the results of PTH measurements to the clinical management of patients with renal bone disease. Technical differences among currently available PTH assays probably account, at least in part, for discrepancies among published results from different research laboratories. A number of other factors may also contribute, some of which have been cited by our European colleagues in their correspondence. It seems unwarranted, however, to speculate retrospectively and without additional supporting information about the potential role of bone aluminum toxicity as a factor that contributes to the skeletal resistance of PTH that may occur in renal failure.

Most of the published work that describes the relationship between PTH levels in serum or plasma and bone histology in patients with renal disease has been done using the immunoradiometric (IRMA) PTH assay originally developed by Nussbaum *et al.* (1). This assay has been widely used for more than a decade in clinical research and by commercial medical diagnostic laboratories worldwide (Allegra PTH; Nichols Institute Diagnostics, San Juan Capistrano, CA). It remains the reference standard for the noninvasive assessment of renal osteodystrophy because abundant supportive bone histopathological data are available (2–11). A similar abundance of clinical and laboratory information has generally not been obtained in studies that have used other PTH-IRMAs to assess patients with renal bone disease.

A new, third-generation PTH-IRMA has recently been introduced (12, 13). Although the relevant characterization data have not been published until now, the detection system used by Fournier *et al.* seems to be very similar to that used in the studies by us (12) and by Slatopolsky *et al.* (13). This kind of PTH assay specifically detects full-length, biologically active 1–84 PTH and fails to cross-react with large aminotermally truncated PTH-derived peptides such as 7–84 PTH that are retained in the plasma of patients with renal failure and are detected by the Nichols' PTH-IRMA (14, 15). The new IRMAs have the potential, therefore, to improve both precision and accuracy in the laboratory diagnosis of renal osteodystrophy.

Despite such considerations, the relationship between serum or plasma PTH levels as determined by third-generation PTH-IRMAs and the underlying histological subtype of renal osteodystrophy has yet to be examined. Such information is essential to critically evaluate the potential use of these new methods for diagnosing and monitoring the evolution of renal bone disease. Until the data become available, guidelines about the concentrations of PTH in serum or plasma that correspond to specific histological lesions of bone in patients with renal osteodystrophy must rely on available published information. Extrapolation of results obtained using new PTH-IRMAs by comparing them to data obtained using previously developed PTH-IRMAs is insufficient to establish their value as a clinical diagnostic tool.

The development of more precise and accurate methods for measuring PTH and PTH-derived peptide fragments in serum or plasma is likely to provide additional insight into the physiology and pathophysiology of parathyroid gland function and bone metabolism in patients with chronic renal failure. Such work may ultimately lead to improvements in diagnostic precision and more refined guidelines for clinical management. Future recommendations should be founded, however, on abundant biochemical and histopathological data obtained from patients who have been well-characterized demographically. Only then can the value of these technical advances in assay methodology be fully determined.

William G. Goodman, Markus R. John, Harald Jüppner, and Isidro B. Salusky
Departments of Pediatrics and Medicine (W.G.G., I.B.S.),
University of California at Los Angeles School of Medicine, Los Angeles, California 90095; and Endocrine Unit, Massachusetts General Hospital and Harvard Medical School (M.R.J., H.J.), Boston, Massachusetts 02114

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Received January 22, 2001. Address correspondence to: Harald Jüppner, M.D., Endocrine Unit, Massachusetts General Hospital, Wellman 501, Boston, Massachusetts 02114.

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What Are “Normal” Testosterone Levels for Women?

To the editor:

The cross-sectional study by Laughlin *et al.* (1), comparing endogenous sex hormones levels with hysterectomy and oophorectomy status, chronological age, and years since menopause in older women, reinforces the possible adverse endocrine sequelae of oophorectomy in older women. However, we question some aspects of the data analysis and, hence, the clinical significance and interpretation of some of the said findings.

The inference from the manuscript is that, in women with intact ovaries, there is an increase in total testosterone levels after the time of menopause and with increasing age reaching premenopausal levels concomitant with falling androstenedione levels. That testosterone should rise while androstenedione falls is incongruous because adrenal androgen precursor levels fall linearly with age (2) and, following the menopause, peripheral conversion of androstenedione becomes a major source of circulating testosterone (3). We would appreciate the authors proposing a hypothesis for this incongruity.

The reason for adjusting total testosterone and bioavailable testosterone for body mass index (BMI) in postmenopausal women is not justified. Because weight may vary significantly with increasing age and years since menopause, and the authors suggest that sex hormone-binding globulin concentrations were inversely related to BMI, one is left to wonder if without adjustment for BMI whether any variation occurred. The authors do not report a relationship between either testosterone or androstenedione and BMI, making the need for the adjustment most curious. Adjustment for BMI is not clinically informative, because the absolute bioavailable circulating levels are the values of physiological significance in terms of direct androgen action and as precursors for extragonadal estrogen biosynthesis in tissues such as bone. Indeed, was

a difference seen for bioavailable testosterone not adjusted for BMI? Non sex hormone-binding globulin-bound testosterone includes albumin-bound testosterone, which may account for up to 20% of total testosterone (4). In view of the age of the subjects, if one is to adjust for BMI, surely one should also adjust for variations in serum albumin. Caution in adjusting for covariates in reporting clinical findings has recently been highlighted (5). Presentation of the unadjusted data from this study would be very informative.

The authors report an assay sensitivity of 0.07 nmol/L for testosterone, however, standard RIAs for testosterone are notoriously inaccurate at the lower end of the female range. We are surprised that such distinct differences were observable in values below 0.5 nmol/L. Furthermore, the actual RIA used for measuring total testosterone is not stated. The most pronounced difference in total testosterone increase was reported to occur between the 6th and 7th decades, yet for the 6th decade there were only 29 women with intact ovaries and seven after oophorectomy. Hence, we caution a 35% increase on a mean value of less than 0.5 nmol/L in so few women becoming a generalizable fact. Furthermore, reporting of values adjusted for BMI gives us little feel for what the actual normal values were, and this is exacerbated in Table 2 in which values were adjusted for both age and BMI.

Although ovarian stromal hypertrophy and hyperplasia may sometimes persist or develop after menopause, probably secondary to elevated LH levels and individual sensitivity, resulting in increased testosterone production (6), other researchers have not found this to be the general rule (7). That “. . . levels in women more than 70 yr of age or 20 yr post menopause were comparable to premenopausal levels” is also questionable. In Fig. 3 in the article, the dotted lines indicate the mean testosterone level for premenopausal women as being between 0.6–0.7 nmol/L. However, according to Sinha-Hikim *et al.* (8), who defined the range of total and free testosterone levels during the normal menstrual cycle in healthy women, the mean testosterone level across the cycle was 1.20 ± 0.69 nmol/L and the mean free testosterone was 12.8 ± 5.59 pmol/L. These levels were measured using an equilibrium dialysis method, and the sensitivity of the total testosterone assay was increased to 0.008 nmol/L. The results from that study are sound and have been used as references in many other studies. Furthermore, these testosterone levels are in agreement with those reported in other literature (9, 10).

If we accept the data of Sinha-Hikim *et al.* (8) as reference levels, then the testosterone levels in the “intact postmenopausal women” in the study by Laughlin *et al.* (1) are, in fact, very low and do not actually increase back to premenopausal levels (1.20 ± 0.69 nmol/L). Although in *Results* the authors state (referencing a 1997 paper) these premenopausal values as being from the same laboratory as used for this study, they are inconsistent with the widely accepted range. Were these also BMI adjusted? Either way, the comparison is not meaningful. Of note, the actual citation for the testosterone levels for premenopausal women in Fig. 3 is that of an article by S. E. Bulun and E. R. Simpson, a study of levels of aromatase cytochrome P450 transcripts in adipose tissue of women. The bioavailable values reported are ~10-fold greater than the normal range for free testosterone. Reporting of free testosterone would have been far more meaningful.

The role of androgens in women is becoming increasingly more recognized and established. Certainly, the use of androgens, particularly testosterone, has been shown to influence life aspects, such as mood, women’s general well being and restoration of sexual desire. However, there is limited data establishing normal androgen values for women of differing ages, to enable us to define those with “androgen deficiency.” It is, therefore, necessary to highlight the incongruities and shortcomings of the paper by Laughlin *et al.* (1), and the need for larger prospective studies to establish the variations in testosterone levels in women with age.

Susan Davis and Jane Tran
The Jean Hailes Foundation
Clayton, Victoria 3168, Australia

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Received April 27, 2000. Address correspondence to: Susan Davis, M.D., Ph.D., Department of Epidemiology and Preventive Medicine, The Jean Hailes Foundation, Melbourne, 291 Clayton Road, Clayton, Victoria 3168, Australia.

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Postmenopausal Testosterone

To the editor:

Drs. Davis and Tran raise important issues that can be summarized to three main areas: 1) the effect of adjusting results for body mass index (BMI); 2) the validity of the testosterone measurements and their relation to premenopausal levels; and 3) a plausible biological explanation for the findings.

1) The effect of adjusting results for BMI.

The relation between hormone levels and menopausal age was analyzed 1) without adjusting for covariates, 2) adjusting for BMI, and 3) adjusting for BMI, smoking, exercise, and alcohol consumption. In the interest of conserving space, only the BMI-adjusted results were presented. The association (or absent association) with age did not differ in unadjusted analyses, and the total and bioavailable testosterone levels were virtually identical to the BMI-adjusted values shown in the figures. The minimal impact of BMI on bioavailable testosterone in this study may reflect the narrow range of BMI among the Rancho Bernardo cohort (95% confidence interval, 23.8–24.5 kg/m²), in whom BMI did not vary across age groups.

2) The validity of the testosterone measurements and their relation to premenopausal levels.

Total testosterone levels were measured by a RIA developed in the steroid research laboratory of Samuel Yen, M.D., during the 1970s using an antibody produced at the University of California–San Diego. An important feature of steroid hormone measurements in this laboratory is sample purification by solvent extraction and celite column chromatography before RIA. The testosterone assay sensitivity of 0.07 nmol/L is comparable with published values (0.03–0.05 nmol/L) for assays using similar sample purification steps (1–3). Because of the high sensitivity and specificity of this method, measured values are lower than direct serum measurements with commercial kits. However, the testosterone levels in this study were similar to levels in other large studies of postmenopausal women using comparable assay methods (1–3).

The authors also question the validity of the testosterone level cited for premenopausal women. Levels for postmenopausal women were compared with a recently published value (0.63 ± 0.23 nmol/L) from the Yen laboratory for 32 women sampled in the early follicular phase of the menstrual cycle (4). A 1979 study (5) using the same assay reported a 24-h mean level of 0.69 ± 0.04 nmol/L for early follicular phase women, therefore, the assay/laboratory has good internal consistency. (The 1973 Judd and Yen article cited by Davis and Tran used an earlier, less specific

assay.) Although the sensitivity of the Sinha-Hikim total testosterone assay is very high, total testosterone levels were apparently measured directly in the dialysate. Equilibrium dialysis would not eliminate cross-reacting substances, which may account for higher (1.04 ± 0.76 nmol/L) early follicular phase testosterone levels with this assay compared with the Yen assay. In our view, steroid hormone assays may yield differing results for a variety of reasons. Direct comparisons are valid only when all samples are measured with the same assay techniques, as was the case in our paper.

Drs. Davis and Tran correctly point out that the finding of an increase in testosterone levels with postmenopausal aging was, in large part, based on lower levels among only 29 intact women in the 50- to 59-yr age range. As stated in the article, “although age-specific levels were based on relatively few women for the youngest decade, the similarity of hormone patterns after stratification by decade and by years since menopause supports the validity of the age-related associations.” Among the intact women, 125 were less than 20, 136 were 20–30, and 119 were more than 30 yr menopausal. Testosterone levels were 25% higher among intact women more than 20 yr postmenopause compared with intact women less than 20 yr postmenopause.

3) A plausible biological explanation for the findings.

The increase in circulating testosterone levels with postmenopausal aging is likely to be ovarian in origin. Testosterone levels did not increase with age among the oophorectomized women, and the metabolism and interconversion of androgens in women is not altered after menopause (6). In recent studies, ovarian vein testosterone levels were related to the degree of stromal hyperplasia in 18 postmenopausal women (7) and correlated positively with age in 52 women aged 42–69 yr (8). Postmenopausal ovarian androgen production is thought to be at least partially gonadotropin driven. Gonadotropin receptors have been identified in the stroma of menopausal ovaries (9), and peripheral steroid levels in postmenopausal women increase after hCG stimulation (10) and decline after administration of long-acting GnRH agonists (11, 12). Thus, stimulation by elevated gonadotropins could account for increased ovarian testosterone synthesis in older women. Although postmenopausal gonadotropin levels tend to decline with advancing age (13), they remain well above premenopausal levels. It is possible that prolonged exposure to high gonadotropin stimulation may up-regulate ovarian responses to LH. To our knowledge, no published studies address this possibility.

The absence of a postmenopausal increase in androstenedione levels with age similar to the increase in testosterone is not surprising. As shown in previous studies and confirmed in this one, the postmenopausal ovary contributes 40% of circulating testosterone, but only 10–15% of circulating androstenedione. Thus, an increase in ovarian androstenedione production would be masked by the many-fold greater adrenal contribution.

Our conclusion that testosterone levels return to the premenopausal range in older, intact postmenopausal women needs clarification. The internal (unpublished) normal laboratory values for this assay are 0.72 ± 0.34, 1.02 ± 0.48, and 0.93 ± 0.38 nmol/L for the early follicular, mid-cycle, and luteal phases, respectively. Because testosterone levels do not undergo cyclic changes in postmenopausal women, our results suggest that older, as well as younger, postmenopausal women are relatively testosterone deficient when compared with younger cycling women, with levels similar to the early follicular phase. We appreciate this opportunity to expand and clarify our findings. Clearly, interpretation of cross-sectional data are subject to error. Prospective studies are needed to define with certainty the pattern of changing testosterone levels in older women.

Gail Laughlin and Elizabeth Barrett-Connor
Department of Family and Preventive Medicine
University of California–San Diego
La Jolla, California 92093-0607

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Received December 18, 2000. Address correspondence to: Elizabeth Barrett-Connor, M.D., Department of Family and Preventive Medicine, University of California–San Diego, Stein Clinical Research Sciences Building, 9500 Gilman Drive, La Jolla, California 92093-0607.

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Long-Term Consequences of Castration in Men

To the editor:

I read with great interest the paper “Long-term consequences of castration in men: lessons from the Skoptzy and the eunuchs of the Chinese and Ottoman courts,” published in December 1999 (1). However, the authors are at fault when, in the last paragraph, they state that the “so-called” castrati singers were a heterogenous group containing only a few singers who had their testes removed or crushed. As I have previously indicated (2), castration was, indeed, performed on a very large scale in Italy specifically to satisfy the ever increasing demand for the unique castrato voices, reaching its peak in the mid 18th century when, according to the great authority on the castrato, Franz Haböck, as many as 4000 castrations were carried out annually (3). It is, unfortunately, not true that only those with extraordinary singing ability were chosen for the procedure. Only a few reached the top opera houses, many sang in ordinary church choirs, and there were many whose mutilation was to no purpose. The castrati nearly all came from poor families in Italy, and fathers permitted the operation in the hope (only too often misplaced) that fame and fortune achieved by being a great castrato would come to them and their poverty-stricken families.

The castrati singers flourished over a century and a half before the medical studies on the Skoptzy or the Chinese and Ottoman eunuchs were carried out. Moreover, despite its popularity in the 18th century, the practice of producing castrati was strictly illegal, according to the canon law of the Roman Church, and every effort was made to keep the identity of the operators and their origin vague. Euphemisms were used to account for the existence of a particular castrato such as disease of the testes or accidental injury—being gored by a wild boar was a favorite reason! It is clear, however, that unlike the groups discussed in the present paper, only the testes were removed; total removal of all the genitalia was never carried out. Only a handful of castrati lived on into the early years of the 20th century, in the Vatican, but 18th century descriptions of the appearances of the castrati include, in addition to the powerful high-pitched singing voice of a strange quality, tallness of stature, smooth beardless face, rounding of the hips, enlargement of the breasts, and a tendency to obesity—all

features we would associated with isolated spontaneous hypogonadism. The sole object was, of course, the preservation of the unbroken voice, and in the only recorded postmortem examination of a castrato the dimensions of the larynx were strikingly small with the vocal cords the length of a female high soprano (4).

With reference to the effect of castration on longevity, a study I carried out on a group of castrated compared with intact singers born at similar times showed that life-span was unchanged (2).

John S. Jenkins
Emeritus Professor of Endocrinology
St. George's Hospital Medical School
SW17 ORE London, United Kingdom

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Citation of “Validation” References for Sphygmocor-Based Estimates of Central Aortic Blood Pressure

To the editor:

Elsheikh *et al.* (1) describe how they attempted to assess central arterial hemodynamics in women with Turner's syndrome.

The whole thrust of their paper suggests that the noninvasive central arterial assessments of “aortic” blood pressure that they adopted have been validated—but no data to support this assertion are actually offered or cited.

Indeed, in the measurements section of their paper, the authors write: “The aortic pulse waveform was derived from the radial pulse waveform by using a validated transfer function (4) implemented in the Sphygmocor software. Measurements using this method have been shown to correspond closely to intraarterially recorded waves (2–4)” (1). However, this is not correct.

Rather, examination of the three references cited from 1989 (2, 3) and 1996 (4) make no mention of the Sphygmocor/BPAS device (PWV Medical, Sydney, Australia) or its radial artery generalized transfer function (GTF). Indeed, none of them show that radial artery pulse waveforms can be used to derive central aortic pulse waveforms noninvasively. One article overviews the basic technique of applanation tonometry (2), and the other two references show how carotid artery tonometry [a technique not used by Elsheikh *et al.* (1)] can provide noninvasive assessments of ascending aortic blood pressure (3, 4).

The literature sometimes cited as having “validated” the radial artery GTF used within the Sphygmocor/BPAS system has recently been over-viewed elsewhere (5). None of the above three articles even feature, because they have nothing to do with the radial artery GTF.

This issue is of importance because in the 7+ yr since the report describing the original radial artery GTF was published (6), and the U.S. Patent for the technique was granted (7), there has been an absolute paucity of validation work with the approach reported in the scientific literature. This lack of validation studies is particularly noticeable when the measurements have to be calibrated noninvasively, as in the study by Elsheikh *et al.* (1). Indeed, what little “validation” data for noninvasive use of the Sphygmocor are available in the literature, based on 20 patients (8), suggests the errors associated with the method for the estimation of central aortic blood pressure to be substantial. In fact, these errors are much greater than those permitted by the American Association for Medical Instrumentation under guidelines endorsed by the U.S. Food and Drugs Administration for noninvasive blood pressure measuring devices (5, 9). This has led to the conclusion that, at this time, there

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Address correspondence and requests for reprints to: Eldon D. Lehmann, Department of Imaging (MR Unit), National Heart and Lung Institute, Imperial College of Science, Technology and Medicine, Sydney Street, London SW3 6NP, United Kingdom. E-mail: info-www@2aida.org.

Received February 29, 2000. Address correspondence to: John S. Jenkins, M.D., 40 Hampstead Way, NW11 7JL London, United Kingdom. E-mail: john.jenkins6@which.net.

are no accurate or well validated methods for measuring blood pressure noninvasively *in vivo* in the aorta (10).

Notwithstanding this, driven it would seem by commercial claims (7) rather than evidence-based medicine, the Sphygmocor GTF approach now is being tried in all manner of disease states completely unrelated to the 14 subjects who provided data at cardiac catheterization for the original generation of the radial artery GTF (6). In this respect, even if one believes the claims that a single radial artery GTF can be used accurately and robustly to measure central aortic blood pressure noninvasively in all subjects of all ages, heights, weights, and blood pressures, both on and off medication (claims that are disputed because no evidence has, in fact, been published in the peer-reviewed literature), it seems completely implausible that this will work for all disease states as well.

Connected with this, a recent query about where is the evidence that the Sphygmocor system can be used to accurately and noninvasively predict central aortic blood pressure in patients with another endocrine disorder (diabetes; Ref. 11) was not able to offer any evidence or data to support the claims that were being made (12). Furthermore, a search on Medline up to September 2000 using "Sphygmocor" and "sphygmocardiography" as search terms confirms the absolute paucity of validation data that are available in the literature for the technique.

Other researchers have noticed this as well and have independently raised separate fundamental concerns about the use of the method, especially for the determination of the augmentation index (13–15) and central aortic blood pressure (16).

Unfortunately, citing papers from elsewhere in the field, which have not involved the Sphygmocor's radial artery GTF, as "validation" references, may make it appear on a cursory inspection that there have, in fact, been more validation studies using the device than are really the case.

Given all the above, Elsheikh *et al.* (1) may wish to exercise caution in making claims about the "validity" of the noninvasive approach they have adopted, which at present remains completely unproven, especially in endocrine disease.

Eldon D. Lehmann

Department of Imaging (MR Unit)

National Heart and Lung Institute

Imperial College of Science, Technology and Medicine
London SW3 6NP, United Kingdom

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Homocysteine, Folate, Vitamin B12, and Transcobalamins in Patients Undergoing Successive Hypo- and Hyperthyroid States

To the editor:

In the March 2000 issue of this journal, Lien *et al.* (1) reported a transient increase in both plasma homocysteine and serum cholesterol during short-term iatrogenic hypothyroidism, which may confer increased cardiovascular risk. The observations of Lien *et al.* (1) are in accordance with the results of our previous preliminary study (2).

We now repeat plasma homocysteine, serum folate, and vitamin B12 in patients with successive hypo- and hyperthyroid states. Cobalamin-binding proteins, transcobalamins, were also determined to explain changes in vitamin B12 concentrations. Forty-five patients [age, 44.5 ± 12.3 yr (23–78); sex ratio M/F, 11/34] who had undergone total thyroidectomy for well-differentiated thyroid carcinoma were studied 4 weeks after the withdrawal of thyroidal hormone therapy and then 14 weeks after the resumption of treatment to suppress the thyrotropin concentration. Hypo- and thyrotoxic states were evidenced by TSH concentrations (Table 1). Total EDTA-plasma homocysteine, serum folate, cobalamin, serum total B12 binding capacity, apo-haptocorrin, and apo-transcobalamin II were measured by methods described previously (4).

Homocysteine concentrations were significantly higher in hypo- than in hyperthyroid state (mean increase, 5.3 ± 4.6 $\mu\text{mol/L}$; Table 1). Furthermore, moderate hyperhomocysteinemia was observed for 10 of 45 patients (22%) with hypothyroidism [range, 17.5–27.2 $\mu\text{mol/L}$; reference range, 6.0–16.0 $\mu\text{mol/L}$ (5)]. Homocysteinemia was normal in all patients in the hyperthyroid state. On univariate analysis, homocysteine was inversely related to folate ($\text{Rho}, -0.33; P = 0.02$). Our results suggested that folate levels may account in determining homocysteine as we confirmed the observation (1) of a moderate decline in serum folate in hypothyroid state, with a P value at the limit of significance ($P = 0.07$). Vitamin B12 was significantly higher in the hypothyroid state than in the hyperthyroid state, as found by Lien *et al.* (1). Transcobalamin levels were determined attempting to explain changes in vitamin B12 concentrations. Except in two cases, apo-haptocorrin was low in all patients in the two states whereas apo-transcobalamin II was not significantly decreased in hypothyroid patients. Vitamin B12 was not correlated to transcobalamins. We concluded that the increased vitamin B12 observed in hypothyroid state could not be explained by changes in transcobalamins.

Lien *et al.* (1) have compared the results at 2-week intervals during two phases. We compared the results between two states, hypo- and hyperthyroidism, in a larger cohort of patients. The increase in homocysteine concentrations during hypothyroidism may be explained by changes in folate status and also by modifications in enzymes involved in homocysteine metabolism, distribution or clearance (1, 6), and/or by concurrent changes in renal function (1). Changes in activities of 5,10-methylenetetrahydrofolate reductase and methionine synthase have been reported during both hyper- and hypothyroid states in an animal model (7). Data about normalization of hyperhomocysteinemia with levothyroxine are conflicting. Normalization was obtained after 3–9 months in a study of 14 patients (8), but failed after 2 months in a cohort of 14 patients (9).

In conclusion, homocysteine was increased in 22% of our patients in the hypothyroidism stage. This mild hyperhomocysteinemia was rather explained by a modification of folates status, with a mild decrease of blood concentration and a negative correlation between folates and homocysteinemia, than by a modification of vitamin B12 status and transport.

Received November 12, 2000. Address correspondence to: Françoise Barbé, Laboratoire de Biochimie A, Centre Hospitalier Universitaire de Nancy, Hôpital de Brabois—Hôpital d'Adultes, Rue du Morvan, 54511 Vandoeuvre-les-Nancy, France. E-mail: f.barbe@chu-nancy.fr.

TABLE 1. Homocysteine, folate, vitamin B12, and transcobalamin results in patients undergoing successive hypo- and hyperthyroid states

	n	Hypothyroid state	Hyperthyroid state	P	Reference range
Serum TSH (mIU/L)	45	83.9 ± 52.5	0.03 ± 0.04	<0.001	0.25–4.0
Serum T ₄ (pmol/L)	26	3.3 ± 1.5	19.5 ± 4.6	<0.001	11.0–25.0
Plasma homocysteine (μmol/L)	45	12.4 ± 5.8	7.1 ± 3.5	<0.001	6.0–16.0
Serum folate (nmol/L)	24	13.4 ± 6.0	15.5 ± 6.4	0.075	6.0–36.0
Serum vitamin B12 (pmol/L)	24	385 ± 102	313 ± 71	<0.001	180–810
Serum total B12 binding capacity (pmol B12/L)	27	464 ± 70	477 ± 67	0.35	
Serum apo-haptocorrin (pmol B12/L)	27	102 ± 24	102 ± 33	0.98	148–627
Serum apo-transcobalamin II (pmol B12/L)	27	362 ± 57	374 ± 50	0.069	295–1328

Results are expressed as the mean ± SD. Paired *t* test was used to evaluate plasma and serum data between hypothyroid and hyperthyroid states. Two-tailed at *P* < 0.05 was reported as statistical significance.

Françoise Barbé, Marc Klein, Abalo Chango, Sophie Frémont, Philippe Gérard, Georges Weryha, Jean-Louis Guéant, and Jean-Pierre Nicolas

Departments of Biochemistry A, INSERM XR308 (F.B., A.C., S.F., P.G., J.-P.N.) and INSERM 0014 (P.G., J.-L.G.) and Department of Endocrinology (M.K., G.W.), Centre Hospitalier Universitaire de Nancy, Hôpital de Brabois–Hôpital d'Adultes 54511 Vandoeuvre-les-Nancy, France

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Total Plasma Homocysteine in Hypo- and Hyperthyroidism: Covariations and Causality

To the editor:

There are consistent reports that patients with hypothyroidism have elevated total homocysteine (tHcy) in plasma and that tHcy is reduced following therapy with T₄ (1–5). The withdrawal of thyroid hormone replacement therapy before radiosciintigraphy of thyroidectomized patients provides controlled conditions for the study of changes in tHcy under variable thyroid status. This strategy was adopted in a recent study from our group (5) and in the study on 45 patients reported by Barbé *et al.*

Barbé *et al.* found lower serum folate in the hypothyroid compared with the hyperthyroid state, and they observed the usual inverse relationship between folate and tHcy. From this observation they inferred that the changes in tHcy may be explained by altered folate status or by a modification of the activity of folate-metabolizing enzymes, as has previously been suggested (2). Like Barbé *et al.*, we found lower levels of serum folate during short-term iatrogenic hypothyroidism (5). We observed, however, that the changes in tHcy

concentration during the hypothyroid phase were more strongly associated with changes in serum creatinine than in folate, suggesting a renal mechanism (5).

The covariation between tHcy and serum cholesterol was equally strong, but a mechanistic link between homocysteine and cholesterol metabolism is not readily apparent. The strong relation between tHcy and cholesterol should remind us that covariations certainly do not prove causality.

Thyroid status has a profound influence on a variety of biochemical processes (6–8), of which some may have secondary effects on homocysteine metabolism. For example, thyroid hormones markedly affect riboflavin metabolism, mainly by stimulating flavokinase and thereby the synthesis of flavin mononucleotide and flavin adenine dinucleotide (FAD; Refs. 7 and 8). Conceivably, these metabolic changes may affect homocysteine metabolism, because flavin mononucleotide and FAD serve as cofactors for enzymes involved in the metabolism of vitamin B₆, cobalamin, and folate (9). Among these enzymes, the FAD-dependent methylenetetrahydrofolate reductase should be considered, because this enzyme is recognized as a possible mediator of changes in tHcy level according to riboflavin status (9).

In conclusion, the nice study of Barbé *et al.* provides longitudinal data in a large number of patients and brings strong support that thyroid status is an important determinant of plasma tHcy and affects serum folate levels. However, the question of the mechanism(s) behind hyperhomocysteinemia in hypothyroid patients remains to be elucidated.

Ernst A. Lien, Steinar Hustad, Bjørn G. Nedrebo, Ottar Nygård, and Per M. Ueland
LOCUS for Homocysteine and Related Vitamins
Armauer Hansens hus
University of Bergen
N-5021 Bergen, Norway

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Received January 22, 2001. Address correspondence to: Ernst A. Lien, M.D., Division of Pharmacology, University Hospital of Bergen, N-5021 Bergen, Norway.