Long-Term Effects of Fenretinide, a Retinoic Acid Derivative, on the Insulin-like Growth Factor System in Women with Early Breast Cancer[1]

Andrea Decensi,[2] Harriet Johansson, Rosalba Miceli, Luigi Mariani, Tiziana Camerini, Elena Cavadini, Maria Gaetana Di Mauro, Antonina Barreca, Aliana Guerrieri Gonzaga, Silvia Diani, Maria Teresa Sandri, Giuseppe De Palo, and Franca Formelli

Division of Chemoprevention [A. D., A. G. G., S. D.], Laboratory Medicine Unit [H. J., M. T. S.], European Institute of Oncology, 20141 Milan; Unit of Medical Statistics and Biometry [R. M., L. M.], Unit of Controlled Clinical Trials [T. C.], Chemoprevention Unit [F. F., E. C.], Division of Clinical Oncology [M. G. D. M., G. D. P.], Istituto Nazionale Tumori, 20133 Milan; and Department of Endocrinology and Metabolic Sciences, University of Genoa, 16132 Genoa [A. B.], Italy

Abstract

High insulin-like growth factor-I (IGF-I) levels are associated with an increased risk of breast cancer in premenopausal women. Because the synthetic retinoid fenretinide showed a beneficial effect on second breast cancers in premenopausal women in a Phase III trial, we studied its long-term effects on IGF-I levels. We measured, at yearly intervals for up to 5 years, the circulating levels of IGF-I, IGF binding protein (BP)-3, and their molar ratio in 60 subjects ≤50 years of age and 60 subjects >50 years of age allocated either to fenretinide or no treatment. In women ≤50 years of age, measurements of IGF-II, IGFBP-1, and IGFBP-2 were also performed. The associations between biomarkers and drug or metabolite plasma concentrations were also investigated. All biomarkers were relatively stable over 5 years in the control group. Compared with controls and after adjustment for baseline, treatment with fenretinide for 1 year induced the following changes: IGF-I, −13% [95% confidence interval (CI), −25 to 1%] in women ≤50 years of age and −3% (95% CI, −16 to 13%) in women >50 years of age; IGFBP-3, −4% (95% CI, −12 to 6%) in both age groups; IGF-I:IGFBP-3 molar ratio, −11% (95% CI, −22 to 1%) in women ≤50 years of age and 1% (95% CI, −11 to 16%) in women >50 years of age. These effects were apparently maintained for up to 5 years, although fewer samples were available as time progressed. No change in other IGF components was observed. Drug and metabolite concentrations were negatively correlated with IGF-I and IGF-I:IGFBP-3 molar ratio in women ≤50 years of age. Fenretinide induces a moderate decline of IGF-I levels in women ≤50 years of age. The association between IGF-I change and the reduction of second breast cancers in premenopausal women warrants further study.

Introduction

IGF[3]-I is a key regulator of proliferation and apoptosis in normal and malignant cells, including breast cancer (1). IGFBP-3 binds nearly 80% of circulating IGFs and influences cell proliferation by regulating binding of IGFs to the IGF receptors (2). Tissue IGF bioactivity is also regulated by circulating levels of at least five other IGFBPs (3).

A positive association between plasma IGF-I levels and subsequent risk of premenopausal breast cancer, prostate cancer, and colorectal cancer has been reported recently in large prospective studies (4–7). For breast cancer, although postmenopausal women with IGF-I levels in the higher quintile had a RR of 0.85, premenopausal women 50 years or age or younger with IGF-I levels in the top tertile had a RR of 4.6 (95% CI, 1.8–12) of developing subsequent breast cancer compared with women in the lowest tertile (6). The RR increased further after adjustment for IGFBP-3 levels (6). A recent prospective study by Toniolo *et al.* (8) also indicated an association, although of lower magnitude, between circulating IGF-I and breast cancer risk in premenopausal but not postmenopausal women. These findings suggest that lowering IGF-I availability might contribute to the reduction of breast cancer risk in young women.

We noted previously in a subgroup of women participating in a secondary prevention trial that 1 year treatment with fenretinide, a synthetic derivative of retinoic acid, affected plasma IGF-I levels differently depending on age, with a statistically significantly greater reduction being found in women ≤50 years of age compared with older women (9, 10). Interestingly, in a *post hoc* analysis of a Phase III trial in nearly 3000 patients, fenretinide was associated with a 35% relative reduction in the incidence of contralateral and ipsilateral breast cancers in premenopausal women (mostly ≤50 years of age), whereas a trend to an increased number of contralateral cancers was observed in postmenopausal women (11). Recently, we also showed in an *in vitro* study that inhibition of breast cancer

Received 3/12/01; revised 7/27/01; accepted 8/15/01.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by NIH National Cancer Institute Grant CA-72286, a contract from the Italian Foundation for Cancer Research (Fondazione Italiana per la Ricerca sul Cancro), and a grant from the Italian Association for Cancer Research (Associazione Italiana Ricerca sul Cancro).
[2] To whom requests for reprints should addressed, at Division of Chemoprevention, European Institute of Oncology, via G. Ripamonti 435, 20141 Milan, Italy. Phone: 39-0257489861; Fax: 39-0257489809; E-mail: andrea.decensi@ieo.it.

[3] The abbreviations used are: IGF, insulin-like growth factor; IGFBP, IGF binding protein; RR, relative risk; CI, confidence interval; 4-MPR, *N*-(4-methoxyphenyl)retinamide.

cell growth by fenretinide is partly mediated by down-regulation of the IGF system (12).

As a first step toward validating IGF-I as a putative surrogate end point biomarker according to Prentice criteria (13), we wished to confirm and extend our previous findings on the effects of fenretinide on the IGF system. We are therefore reporting the results in a different and larger series of randomly selected subjects within the Phase III trial. Specifically, the objectives of the present study were to examine: (*a*) the effect of 1 year treatment with fenretinide on IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio in women ≤50 and >50 years of age; (*b*) the stability of the biomarkers over the 5-year intervention period; (*c*) the long-term effects of fenretinide on other IGF components (IGF-II, IGFBP-1, IGFBP-2, and the molar ratio of IGF-I and IGF-II over the sum of IGFBP-1, IGFBP-2, and IGFBP-3) in women ≤50 years of age; (*d*) the association between the biomarker levels and drug or metabolite 4-MPR plasma concentrations in the fenretinide-treated women. The findings could help in selecting the appropriate IGF components and the specific time intervals for a larger study on the role of IGF-I as a surrogate end point biomarker of second breast cancers among all of the subjects with available plasma aliquots who took part in the Phase III study.

Subjects and Methods

Subjects and Treatment. The present series of patients was selected from a Phase III chemoprevention trial, the design and main results of which have been reported recently (11). Briefly, from March 1987 to July 1993, the 2972 women enrolled met the following criteria: ages between 30 and 70 years, treatment for stage I breast cancer (T_1–T_2 N_0) or ductal carcinoma *in situ* with curative surgery, and disease free at the time of study entry. Women were randomly assigned to receive no treatment or fenretinide (R. W. Johnson Pharmaceutical Research Institute, Springhouse, PA), 200 mg p.o. daily for 5 years (two capsules at dinner), with a 3-day drug holiday at the end of each month. No woman was treated with tamoxifen or hormone replacement therapy during the trial. Blood samples were collected in all subjects before starting treatment and in most of them after 1 year treatment. Thereafter, blood was collected on a yearly basis in all available subjects in the treated arm and in randomly selected subjects in the control arm. Measurements of plasma levels of fenretinide, its main metabolite 4-MPR, and retinol were performed as described previously (14).

For the present study, we randomly selected a subgroup of 60 women ≤50 years of age (30 in the fenretinide arm and 30 in the control arm) and 60 women >50 years of age (30 in each arm) among those who had plasma aliquots at least at two time points, *i.e.*, baseline and at 1 year of study. Despite slight misclassifications (Table 1), ≤50 years of age was used as a surrogate of premenopausal status to be consistent with our previous study (9), where age was a better modifier than was menopausal status, of the effect of fenretinide on IGF-I change. However, the results of the present study proved to be no different when subjects were categorized according to menopausal status (data not shown). To minimize the risk of a selection bias, women were studied regardless of their follow-up duration and treatment outcome. This implied a substantial loss of observation because of occurring events or dropouts with time. Two control subjects, one in each age group, could not be included in the analyses because of an insufficient plasma amount at baseline. To reduce variability, only samples collected within 24 h from the fenretinide intake were analyzed. After this selection, a total of 270 samples in women ≤50 years of age and 283 samples in women >50 years of age were available for analysis.

Table 1 Main subject characteristics

	Age ≤50		Age >50	
	Fenretinide (n = 30)	Control (n = 30)	Fenretinide (n = 30)	Control (n = 30)
Age, years (mean ± SD)	45.2 ± 4.0	44.3 ± 3.9	56.8 ± 4.5	59.6 ± 5.2
Pre/postmenopausal status	28/2	30/0	4/26	2/28
Tumor stage: pT_1/pT_2	26/4	25/5	25/5	25/5
Quadrantectomy/mastectomy	25/5	26/4	26/4	22/8
Ductal/lobular/other histology	22/5/3	22/8/0	24/6/0	24/5/1
Years from diagnosis to randomization				
<1/1–3/>3	23/4/3	22/6/2	23/3/4	22/5/3

Assay Methods for IGFs and IGFBPs Measurements. Blood samples were drawn between 9 a.m. and 3 p.m., mostly before lunch. Although this relatively large time interval may represent an additional source of variability in IGFs levels, it was considered acceptable because no significant daily variations or influence of food intake have been observed for IGFs (2). Plasma aliquots obtained using heparin were separated by centrifugation and stored at −80°C until assayed. Plasma concentration of total IGF-I was determined by a RIA method using commercially available kits purchased from Biosource (Fleurens, Belgium). The sensitivity of the assay was 0.02 nmol/l; intra- and interassay coefficients of variation for IGF-I were 6 and 7.5%, respectively. IGF-II was measured by a double antibody RIA using a monoclonal antibody provided by Sera-Lab (Crawley Down, Sussex, United Kingdom); the secondary polyclonal antibody was provided by Calbiochem (La Jolla, CA). Human recombinant ^{125}I-labeled IGF-II was provided by Amersham Pharmacia Biotech (Milan, Italy). The standard curve was performed using recombinant IGF-II from GroPep Pty Ltd. (Adelaide, Australia). The intra- and interassay coefficients of variation were, respectively, 3.7 and 6.9% (mean concentration, 0.523 nmol/l). Acid/ethanol extraction was performed before IGF assays to remove interfering binding proteins, according to the method described previously (15). Although low molecular weight binding proteins may not be completely removed by this procedure, previous studies showed that addition of IGFBP-1 and IGFBP-2 does not significantly interfere with the tracer. However, chromatographic analysis in acid conditions was performed in some samples to confirm effective removal. Serum IGFBP-3 was measured by RIA using commercially available kits provided by Diagnostic System Laboratories (Webster, TX). The sensitivity of the assay was 0.012 nmol/l; the intra- and interassay coefficients of variations were 4.0 and 5.5%, respectively. Serum IGFBP-1 was measured by a two-site immunoradiometric assay provided by Diagnostic Systems Laboratories, Inc. The sensitivity of the assay was 0.013 nmol/l; the intra- and interassay coefficients of variation were 4 and 5.0%, respectively. Serum IGFBP-2 was determined by a double-antibody RIA using a commercially available kit provided by Diagnostic Systems Laboratories Inc. The sensitivity of the assay was 0.016 nmol/l; the intra- and interassay coefficients of both was 6%. All data were expressed in nanomolar concentrations to calculate the IGF-I:IGFBP-3 molar ratio, inasmuch as IGF-I levels adjusted for IGFBP-3 are associated more strongly with breast cancer risk than IGF-I levels alone in premenopausal women ≤50 years of age (6). Moreover, the sum of polypeptide ligands over IGFBPs (IGF-I

Table 2 Baseline IGF geometric means (nmol/l, and 95% CI) in women ≤50 and >50 years of age allocated to fenretinide or no treatment (control)

	Age ≤50 years		Age >50 years	
	Fenretinide (n = 30)	Control (n = 29)	Fenretinide (n = 30)	Control (n = 29)
IGF-I	24.1 (20.4–28.6)	25.6 (22.0–30.0)	14.6 (12.3–17.4)	13.8 (11.5–16.6)
IGFBP-3	101.9 (90.5–114.7)	102.1 (92.2–113.1)	73.7 (69.2–78.6)	79.0 (72.1–86.5)
IGF-I:IGFBP-3	0.2 (0.2–0.3)	0.3 (0.2–0.3)	0.2 (0.2–0.2)	0.2 (0.2–0.2)
IGF-II	65.1 (57.1–74.2)	69.2 (62.4–76.8)		
IGFBP-1	0.9 (0.7–1.2)	1.1 (0.9–1.5)		
IGFBP-2	9.1 (7.5–11.0)	9.7 (8.1–11.7)		
Free IGFs[a]	0.8 (0.7–0.9)	0.9 (0.8–0.9)		

[a] (IGF-I + IGF-II):(IGFBP-1 + IGFBP-2 + IGFBP-3) molar ratio.

+ IGF-II:IGFBP-1 + IGFBP-2 + IGFBP-3) was calculated to obtain the nanomolar concentrations of free, biologically active ligands (free IGFs), using the formula described previously (16).

Statistical Methods. From previous studies (9, 10) in women ≤50 years of age, we assumed a mean ± SD of 20 ± 6 nmol/l for baseline IGF-I, a 15% relative reduction in the mean level after 1 year of treatment with fenretinide, and a SD of the difference of 4 nmol/l. A sample of 30 subjects per arm was estimated to have an 80% power to detect such a reduction with a two-tailed t test (or ANOVA) at a 5% significance level. An equal number of subjects was selected among women >50 years of age. In all of the analyses, IGF measurements were log-transformed to approximate a Gaussian distribution. Descriptive analysis was carried out by computing the geometric means and the 95% CIs for baseline biomarker levels as well as for the ratios between baseline and the following time windows: 7–18 months (year 1); 19–30 months (year 2); 31–42 months (year 3); 43–54 months (year 4); and 55–66 months (year 5). When multiple measurements were available in a given year, the measurement nearest to the midpoint was chosen. A 95% CI for the ratio that excludes the value of 1 (this latter corresponding to a lack of difference) is roughly equivalent to a significant t test at the 5% level. Relative percent changes can be obtained by taking the absolute value of (1 − ratio) × 100.

The comparison of the mean biomarker values obtained at year 1 in the two treatment arms was performed by means of covariance analysis. The factors included in the analysis were: baseline biomarker level, treatment, and, limited to IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio, age (≤50, >50 years), and treatment by age interaction. The assumption of parallelism in the regression between year 1 and baseline level in the two treatment arms was first verified.

To assess the long-term effects of treatment, time since randomization, and subject's age on mean biomarker levels, all measurements available in each subject up to 5 years were considered (on average, 3.7 measurements/subject). The effects were tested by using linear mixed models (17), taking into account the correlation existing among repeated measurements within the same subject. After exploring the correlation structure with variograms (18), a compound symmetry covariance matrix was adopted for all biomarkers. Time factor was modeled using a linear spline with a knot at 13 months, which allows for a different pattern of variation between the first and subsequent years of study. Other factors included in the models were: (*a*) treatment, age (≤50, >50 years), and, limited to IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio, all interactions between time, treatment, and age; and (*b*) treatment and the interactions between treatment and time for IGF-II, IGFBP-1, IGFBP-2, and total ratio of free IGFs.

The association between biomarker levels and drug or 4-MPR plasma concentrations was assessed in the fenretinide-treated group using the Spearman correlation coefficient (r_S). The analyses were performed by means of SAS software (19).

Results

Subject Characteristics and Pretreatment IGF Values. The main characteristics of the 120 subjects are described in Table 1. The two treatment arms were well balanced and comparable with the whole study cohort (11). Among patients ≤50 years of age, two women in the fenretinide group were postmenopausal at randomization, whereas among those >50 years, four women in the fenretinide and two in the control group were premenopausal.

Mean IGF values at baseline are reported in Table 2. Within each age group, the two treatment arms had comparable mean values. However, the levels of IGF-I and IGFBP-3 were much lower in women >50 years than in the younger subjects, both variables exhibiting an exponential decline with age (data not shown).

On Treatment IGF Values. The 5-year course of the mean values of IGF-I, IGFBP-3 and IGF-I:IGFBP-3 molar ratio in the two treatment arms according to age is illustrated in Fig. 1. Compared with baseline, a trend toward a reduction in IGF-I and IGF-I:IGFBP-3 molar ratio was observed in women ≤50 years in the fenretinide arm. A remarkable stability over time was noted in the control group.

The ratios between baseline and follow-up measurements of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio are reported in Table 3 for descriptive purposes. No relevant biomarker change occurred in the control group during time. In the fenretinide group, the mean changes at year 1 relative to baseline in women ≤50 and >50 years were, respectively: (*a*) IGF-I: −16 and −8%; (*b*) IGFBP-3, −12% and −4%; and (*c*) IGF-I:IGFBP-3 molar ratio, −4% and −5%.

The relative changes of IGF-II, IGFBP-1, IGFBP-2, and free IGFs among women ≤50 years are reported in Table 4. No significant variation was noted during the 5-year intervention period in both arms. Consequently, no further study of these biomarkers was undertaken in women >50 years.

The results of covariance analysis for the biomarker changes at year 1 after adjustment for baseline values are summarized in Table 5. Compared with no treatment, fenretinide induced a change of −13% (95% CI, −25 to 1%) of IGF-I in women ≤50 years and of −3% (95% CI, −16% to 13%) in women >50 years. The change of IGFBP-3 was −4% (95% CI, −12% to 6%) in both age groups; the change of IGF-I:IGFBP-3 molar ratio associated with fenretinide was −11% (95% CI,

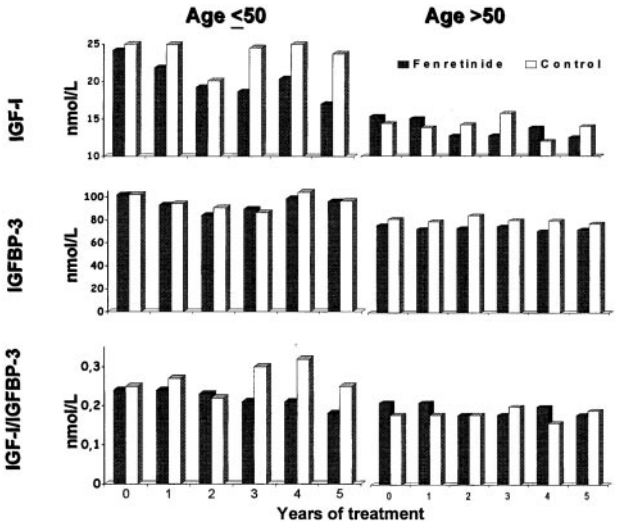

Fig. 1. Mean levels of IGF-I (*top*), IGFBP-3 (*middle*), and IGF-I:IGFBP-3 molar ratio (*bottom*) in women ≤50 and >50 years of age treated for 5 years with fenretinide or no treatment (control).

−22% to 1%) in women ≤50 years and 1% (95% CI, −11% to 16%) in women >50 years. However, for none of the three markers was the effect of fenretinide significantly different in the two age groups, as indicated by the *P*s for the interaction between treatment and age. For the remaining biomarkers, the reduction was generally low, with the exception of IGFBP-I, for which a nonsignificant increase was observed in the fenretinide arm.

Long-Term Treatment Effects. The predicted mean values of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio according to treatment, age, and time since randomization are shown in Fig. 2. The predicted values were derived from the mixed models as described in "Subjects and Methods." A reduction in IGF-I and IGF:IGFBP-3 molar ratio was evident at year 1 in the fenretinide arm, whereas no change occurred in the control group ($P = 0.009$ and $P = 0.044$, respectively, for the interaction between treatment and time since randomization). After year 1, the mean biomarker levels were stable in both arms. The levels of IGFBP-3 were not affected by treatment but showed a different behavior over time in the two age groups as a result of a decline in IGFBP-3 levels during year 1 among women ≤50 years and not among older women ($P = 0.002$ for the interaction between age and time since randomization). As for IGF-II, IGFBP-1, IGFBP-2, and free IGFs, no significant treatment effect or time by treatment interaction were observed in women ≤50 years (data not shown).

Association between Drug Plasma Concentrations and IGF Components. In general, the estimated correlation coefficients (r_s) showed weak associations between drug or metabolite plasma concentrations and IGF components in both age groups. However, a significant negative association was observed between fenretinide plasma concentrations and IGF-I ($r_s = -0.24$; $P = 0.008$) and IGF-I:IGFBP-3 molar ratio ($r_s = -0.22$; $P = 0.017$) in women ≤50 years. Likewise, 4-MPR plasma concentrations were negatively associated with IGF-I ($r_s = -0.36$; $P < 0.001$), IGF-I:IGFBP-3 molar ratio ($r_s = -0.32$; $P = 0.001$), and IGF-II ($r_s = -0.22$; $P = 0.017$).

Discussion

There is growing evidence that IGF-I is involved in the development of normal breast tissue (1, 20) and may also be associated with breast cancer formation in premenopausal women (6, 21). Our previous pilot study showed that the modulation of circulating IGF-I levels by fenretinide administered for 1 year differed according to age or menopausal status, a greater reduction being shown in women ≤50 years of age (9, 10). This pattern is consistent with the clinical results of a Phase III trial, where the relative risk of second breast cancers was 35% lower in premenopausal women treated with fenretinide compared with those who received no treatment, whereas no beneficial effect or even a slight trend toward an increase in contralateral breast cancer was noted in women >55 years in the fenretinide arm (11). In the light of the potential ability of IGF-I to serve as a surrogate biomarker for breast cancer prevention, we have extended our study to other IGF components and assessed their long-term behavior within the fenretinide trial.

Compared with controls and adjusting for baseline values, a borderline significant decline of 13% in IGF-I and of 11% in IGF-I:IGFBP-3 molar ratio was observed after the first year of fenretinide treatment in women ≤50 years, whereas no change was noted in women >50 years. These effects were durable throughout the 5-year study period. Consistently, drug and metabolite plasma concentrations were negatively correlated with IGF-I and IGF-I:IGFBP-3 molar ratio in women ≤50 years. Conversely, no change in IGF-II, IGFBP-1, IGFBP-2, and total free IGFs was observed in both arms during the study period.

Given the positive association of circulating IGF-I and IGF-I:IGFBP-3 molar ratio with mammographic density (21) and with subsequent risk of breast cancer (6) in women ≤50 years, our data lead to the hypothesis that fenretinide might at least in part reduce breast cancer risk in this age group through interference with the IGF pathway. Because these effects can be achieved as early as after 1 year of study and tend to plateau thereafter, and because all biomarkers in the control group were relatively stable over time, circulating IGF-I and the IGF-I:IGFBP-3 molar ratio measured after 1 year of intervention may thus qualify as an early surrogate end point in breast cancer prevention trials in premenopausal women.

The magnitude of the changes induced by fenretinide on IGF-I and on the IGF-I:IGFBP-3 molar ratio in women ≤50 years was of moderate entity, a mean −13% change (95% CI, −25 to 1%) of IGF-I being the largest effect induced by fenretinide. This may be a consequence of the substantial interindividual variability in drug and IGFs levels, which is at least in part genetically driven (22). However, a moderate effect may not be undesirable in a preventive context, given the long-term positive association between IGF-I decline and aging (23, 24) and the potential therapeutic activity of exogenous growth hormone and IGF-I against heart failure (25). Thus, a moderate yet durable decline in IGF-I might attain a meaningful risk reduction in breast cancer without negatively affecting other essential target systems. Notably, in the Nurses' Health Study (6) a 24% difference in unadjusted IGF-I levels between highest and lowest tertiles of premenopausal women ≤50 years was associated with a significantly increased risk of developing breast cancer (RR, 4.6). The RR increased to 7.3 when the analysis was adjusted for IGFBP-3 levels. Likewise, a 35% difference in IGF-I levels was associated with an odds ratio of 2.3 of developing breast cancer in the study by Toniolo *et al.* (8). Thus, moderate differences in IGF-I levels are associated with substantial variations in risk of breast cancer (6). These

Table 3 Relative changes of IGF-I, IGFBP-3, and their molar ratio during the 5 years of intervention

Data are expressed as the mean ratio (and 95% CI) between baseline and yearly measurements.

IGF and year	Age ≤50 years				Age >50 years			
	Fenretinide		Control		Fenretinide		Control	
	n	Geometric mean (95% CI)	*n*	Geometric mean (95% CI)	*n*	Geometric mean (95% CI)	*n*	Geometric mean (95% CI)
IGF-I								
1	25	0.84 (0.75–0.95)	28	0.97 (0.86–1.10)	24	0.92 (0.83–1.01)	28	0.98 (0.88–1.08)
2	21	0.89 (0.78–1.01)	9	0.90 (0.76–1.07)	23	0.85 (0.77–0.93)	15	1.00 (0.80–1.25)
3	17	0.88 (0.79–0.98)	9	0.99 (0.81–1.21)	11	0.89 (0.67–1.19)	8	0.86 (0.63–1.17)
4	14	1.02 (0.88–1.18)	4	0.83 (0.50–1.40)	16	0.82 (0.70–0.95)	5	1.11 (0.69–1.79)
5	10	0.91 (0.72–1.15)	9	0.89 (0.76–1.06)	14	0.94 (0.80–1.09)	9	1.06 (0.78–1.43)
IGFBP-3								
1	25	0.88 (0.80–0.96)	27	0.92 (0.84–1.00)	24	0.96 (0.91–1.01)	28	0.98 (0.93–1.03)
2	21	0.81 (0.73–0.91)	8	0.95 (0.86–1.05)	23	0.99 (0.95–1.04)	15	0.97 (0.90–1.05)
3	17	0.85 (0.77–0.94)	8	0.87 (0.72–1.06)	11	1.00 (0.93–1.07)	8	0.88 (0.83–0.95)
4	14	0.90 (0.77–1.05)	4	0.98 (0.74–1.31)	16	0.97 (0.92–1.02)	5	0.95 (0.82–1.10)
5	10	0.90 (0.80–1.02)	9	0.96 (0.85–1.07)	14	0.97 (0.90–1.06)	9	1.01 (0.91–1.13)
IGF-I:GFBP3								
1	25	0.96 (0.86–1.08)	27	1.08 (0.97–1.20)	24	0.95 (0.88–1.04)	28	1.00 (0.91–1.10)
2	21	1.09 (0.95–1.24)	8	1.00 (0.90–1.12)	23	0.85 (0.77–0.94)	15	1.02 (0.81–1.29)
3	17	1.04 (0.92–1.16)	8	1.22 (1.08–1.39)	11	0.89 (0.66–1.21)	8	0.97 (0.69–1.39)
4	14	1.13 (0.96–1.33)	4	0.85 (0.65–1.10)	16	0.84 (0.73–0.97)	5	1.17 (0.66–2.09)
5	10	1.01 (0.83–1.23)	9	0.94 (0.78–1.12)	14	0.96 (0.82–1.12)	9	1.05 (0.77–1.43)

Table 4 Relative changes of IGF-II, IGFBP-1, IGFBP-2, and total free IGFs ratio during the 5 years of intervention in women ≤50 years of age

Data are expressed as the mean ratio (and 95% CI) between baseline and yearly measurements.

IGF and year	Fenretinide		Control	
	n	Geometric mean (95% CI)	*n*	Geometric mean (95% CI)
IGF-II				
1	25	0.97 (0.87–1.09)	28	1.02 (0.94–1.11)
2	21	1.07 (0.98–1.16)	9	0.94 (0.77–1.16)
3	17	0.94 (0.84–1.05)	9	0.90 (0.72–1.13)
4	14	1.01 (0.86–1.18)	3	0.99 (0.87–1.12)
5	10	0.92 (0.75–1.14)	9	0.93 (0.85–1.02)
IGFBP-1				
1	25	1.06 (0.80–1.42)	28	0.87 (0.68–1.11)
2	21	1.26 (0.91–1.73)	9	0.64 (0.42–0.96)
3	17	1.04 (0.68–1.59)	9	0.70 (0.43–1.14)
4	14	1.26 (0.92–1.72)	4	0.96 (0.57–1.62)
5	10	1.53 (0.82–2.85)	9	0.91 (0.64–1.29)
IGFBP-2				
1	25	1.05 (0.82–1.35)	28	1.06 (0.91–1.24)
2	20	1.10 (0.86–1.41)	9	1.16 (0.94–1.42)
3	15	1.05 (0.82–1.33)	9	1.17 (0.82–1.68)
4	11	1.08 (0.78–1.50)	4	0.84 (0.70–0.99)
5	10	0.94 (0.67–1.31)	9	1.04 (0.75–1.46)
Free IGFs[a]				
1	25	1.04 (0.93–1.17)	27	1.08 (0.99–1.19)
2	20	1.18 (1.06–1.32)	8	1.00 (0.87–1.16)
3	15	1.00 (0.89–1.12)	8	1.11 (0.87–1.41)
4	11	1.12 (0.98–1.27)	3	0.91 (0.84–0.98)
5	10	1.01 (0.83–1.22)	9	0.96 (0.84–1.10)

[a] (IGF-I + IGF-II):(IGFBP-1 + IGFBP-2 + IGFBP-3) molar ratio.

Table 5 Covariance analysis of the relative changes of IGFs and IGFBPs after 1 year of intervention, by age

Data are expressed as the mean ratio (and 95% CI) between fenretinide and control adjusting for baseline values.

	Fenretinide *vs.* Control	*p*
IGF-I		
≤50	0.87 (0.75–1.01)	0.288[a]
>50	0.97 (0.84–1.13)	0.122[b]
IGFBP-3		
≤50	0.96 (0.88–1.06)	0.999[a]
>50	0.96 (0.88–1.06)	0.250[b]
IGF-I/IGFBP-3		
≤50	0.89 (0.78–1.01)	0.170[a]
>50	1.01 (0.89–1.16)	0.274[b]
IGF-II		
≤50	0.96 (0.86–1.08)	0.486[b]
IGFBP-1		
≤50	1.18 (0.87–1.59)	0.287[b]
IGFBP-2		
≤50	0.95 (0.72–1.25)	0.728[b]
Free IGFs[c]		
≤50	0.95 (0.84–1.08)	0.466[b]

[a] *P* for the interaction between treatment and age.
[b] *P* for treatment.
[c] (IGF-I + IGF-II):(IGFBP-1 + IGFBP-2 + IGFBP-3) molar ratio.

figures are consistent with the 13 and 11% durable decline of IGF-I and IGF-I:IGFBP-3 molar ratio being associated with a 35% lower risk of second breast malignancies in premenopausal women in our study (11). On the basis of these findings, we are currently determining the effect of the changes in IGF-I and the IGF-I:IGFBP-3 molar ratio on the risk of second breast malignancy in a large series of women participating in our Phase III trial.

Although the mechanisms underlying the preferential effect of fenretinide in women ≤50 years of age are unclear, several lines of evidence support a permissive role of estrogens on IGF-I synthesis and response to fenretinide: (*a*) endogenous estrogens have been shown to exert a direct regulation on circulating IGF-I synthesis (26, 27). This is supported by our finding that women exhibited an exponential decrease of IGF-I levels with age, at variance with the linear decline observed in postmenopausal women (28); (*b*) the observation that the risk of second breast malignancy is ~3-fold higher in premenopausal

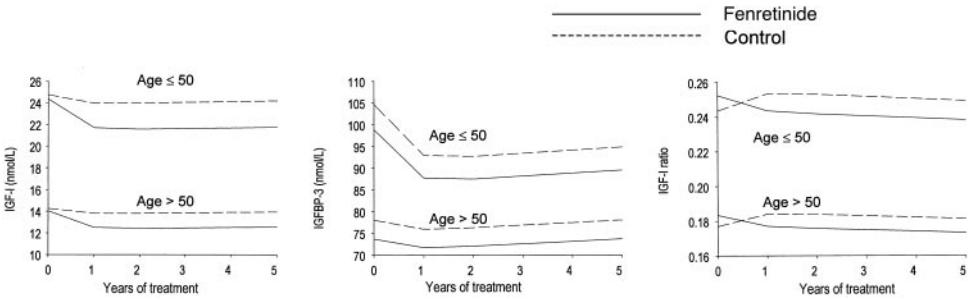

Fig. 2. Mean levels of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio estimated by linear mixed models in women ≤50 and >50 years of age treated for 5 years with fenretinide (*continuous line*) or no treatment (*dashed line*).

women than in postmenopausal women in the control group (11) seems to be consistent with the finding that estradiol sensitizes the normal human breast gland to the proliferative effect of IGF-I in mice xenografts (29); and (*c*) estradiol has been shown to sensitize breast cancer cell lines to the growth-inhibitory effects of retinoids (30, 31), and activated retinoic acid receptors may have antiestrogenic effects by competing directly or indirectly with ER for binding to estrogen response elements (32). Altogether, these findings suggest that the fenretinide-associated reduction of IGF-I levels might lower breast cancer risk in women with adequate circulating estrogen levels. Conversely, the estrogen drop that occurs across menopause might blunt fenretinide activity or even switch its effect toward a partial promoting effect, in line with the notion that cross-regulation between ligands of the steroid receptor superfamily may lead to pleiotropic biological effects, depending upon hormonal context (33). Notably, the trend to a lower incidence of hot flushes observed in an adjuvant trial of fenretinide *versus* placebo in postmenopausal patients receiving tamoxifen (34) might even suggest a partial estrogenic effect of fenretinide. Further studies are clearly necessary to confirm this complex hypothesis.

In the present study, we were unable to confirm our previous observation of a slight, independent increase of IGFBP-3 levels during fenretinide treatment (10). Indeed, IGFBP-3 levels showed a trend to a reduction in both arms. Likewise, no change in IGFBP-3 levels was observed in a study of fenretinide in men with superficial bladder cancer (35). The reason for this discrepancy among studies is presently unclear, although it may at least in part be attributable to differences in age and genetic factors of the populations studied. Interestingly, a recent report (36) describes a polymorphism in the promoter region of IGFBP-3 that appears to influence the effect of retinoids on IGFBP-3 expression. Additional studies are warranted to identify the genetic, hormonal, and lifestyle factors that influence circulating levels of IGFBP-3 (22).

Our data indicate a lack of effect of fenretinide on IGF-II levels in premenopausal women, supporting the concept that circulating IGF-II levels are not under hormonal regulation, as already noted with tamoxifen and other ligands of the steroid receptor superfamily (37). Although the physiological function of circulating IGF-II in adult life remains largely unknown, no significant association with cancer risk has thus far been demonstrated (4, 5). Despite its presence at higher concentrations in the circulation, IGF-II has a higher affinity to the IGFBPs and a lower affinity to type I IGF receptor than does IGF-I, thus playing a less important role than circulating IGF-I (2). Conversely, an increased expression of IGF-II in the stroma surrounding the tumor has been implicated in breast cancer formation (38).

The levels of IGFBP-1 showed a slight tendency to an increase in women ≤50 years of age on fenretinide, although this effect did not reach statistical significance. Qualitatively, this effect is similar to tamoxifen (39). Although the contribution of IGFBP-1 to the total binding activity of circulating IGFs is modest, an increase in the levels of this protein may inhibit the release of IGFs to the target tissues (40). Moreover, high IGFBP-1 levels have been associated recently with a lower risk of colorectal cancer in a prospective study (40, 41). As regards IGFBP-2, no change during treatment was observed in women ≤50 years. Although the role of circulating IGFBP-2 is currently unknown, this protein has been shown to be positively associated with cell proliferation (12, 42).

In conclusion, 5 years of fenretinide treatment induces a moderate decline of circulating IGF-I and IGF-I:IGFBP-3 molar ratio in women ≤50 years of age with early breast cancer. This effect may contribute to the beneficial trend of the retinoid on second breast cancer observed in women in this age group and supports further investigations on the role of plasma IGF-I as a surrogate biomarker.

References

1. Pollak, M. Insulin-like growth factor physiology and cancer risk. Eur. J. Cancer, *36:* 1224–1228, 2000.

2. Yu, H., and Rohan, T. Role of the insulin-like growth factor family in cancer development and progression. J. Natl. Cancer Inst., *92:* 1472–1489, 2000.

3. Hwa, V., Oh, Y., and Rosenfeld, R. G. The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr. Rev., *20:* 761–787, 1999.

4. Chan, J. M., Stampfer, M. J., Giovannucci, E., Gann, P. H., Ma, J., Wilkinson, P., Hennekens, C. H., and Pollak, M. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science (Wash. DC), *279:* 563–566, 1998.

5. Ma, J., Pollak, M. N., Giovannucci, E., Chan, J. M., Tao, Y., Hennekens, C. H., and Stampfer, M. J. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J. Natl. Cancer Inst., *91:* 620–625, 1999.

6. Hankinson, S. E., Willett, W. C., Colditz, G. A., Hunter, D. J., Michaud, D. S., Deroo, B., Rosner, B., Speizer, F. E., and Pollak, M. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet, *351:* 1393–1396, 1998.

7. Giovannucci, E., Pollak, M. N., Platz, E. A., Willett, W. C., Stampfer, M. J., Majeed, N., Colditz, G. A., Speizer, F. E., and Hankinson, S. E. A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol. Biomark. Prev., *9:* 345–349, 2000.

8. Toniolo, P., Bruning, P. F., Akhmedkhanov, A., Bonfrer, J. M., Koenig, K. L., Lukanova, A., Shore, R. E., and Zeleniuch-Jacquotte, A. Serum insulin-like growth factor-I and breast cancer. Int. J. Cancer, *88:* 828–832, 2000.

9. Torrisi, R., Pensa, F., Orengo, M. A., Catsafados, E., Ponzani, P., Boccardo, F., Costa, A., and Decensi, A. The synthetic retinoid fenretinide lowers plasma insulin-like growth factor I levels in breast cancer patients. Cancer Res., *53:* 4769–4771, 1993.

10. Torrisi, R., Parodi, S., Fontana, V., Pensa, F., Casella, C., Barreca, A., De Palo, G., Costa, A., and Decensi, A. Effect of fenretinide on plasma IGF-I and IGFBP-3 in early breast cancer patients. Int. J. Cancer, *76:* 787–790, 1998.

11. Veronesi, U., De Palo, G., Marubini, E., Costa, A., Formelli, F., Mariani, L., Decensi, A., Camerini, T., Del Turco, M. R., Di Mauro, M. G., Muraca, M. G., Del Vecchio, M., Pinto, C., D'Aiuto, G., Boni, C., Campa, T., Magni, A., Miceli, R., Perloff, M., Malone, W. F., and Sporn, M. B. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. J. Natl. Cancer Inst., *91:* 1847–1856, 1999.

12. Favoni, R. E., de Cupis, A., Bruno, S., Yee, D., Ferrera, A., Pirani, P., Costa, A., and Decensi, A. Modulation of the insulin-like growth factor-I system by *N*-(4-hydroxyphenyl)-retinamide in human breast cancer cell lines. Br. J. Cancer, *77:* 2138–2147, 1998.

13. Prentice, R. L. Surrogate endpoints in clinical trials: definition and operational criteria. Stat. Med., *8:* 431–440, 1989.

14. Formelli, F., Carsana, R., Costa, A., Buranelli, F., Campa, T., Dossena, G., Magni, A., and Pizzichetta, M. Plasma retinol level reduction by the synthetic retinoid fenretinide: a one year follow-up study of breast cancer patients. Cancer Res., *49:* 6149–6152, 1989.

15. Daughaday, W. H., Mariz, I. K., and Blethen, S. L. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. J. Clin. Endocrinol. Metab., *51:* 781–788, 1980.

16. Juul, A., Main, K., Blum, W. F., Lindholm, J., Ranke, M. B., and Skakkebaek, N. E. The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. Clin. Endocrinol., *41:* 85–93, 1994.

17. Searle, S. R., Casella, G., and McCulloch, C. E. Variance Components. New York: John Wiley & Sons, 1992.

18. Diggle, P. J., Liang, K. Y., and Zeger, S. L. Analysis of longitudinal data. Oxford: Oxford University Press, 1994.

19. SAS Institute, Inc. SAS/STAT User's Guide, Version 6. Cary, NC: SAS Institute, Inc., 1989.

20. Ng, S. T., Zhou, J., Adesanya, O. O., Wang, J., LeRoith, D., and Bondy, C. A. Growth hormone treatment induces mammary gland hyperplasia in aging primates. Nat. Med., *3:* 1141–1144, 1997.

21. Byrne, C., Colditz, G. A., Willett, W. C., Speizer, F. E., Pollak, M., and Hankinson, S. E. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res., *60:* 3744–3748, 2000.

22. Jernstrom, H., Deal, C., Wilkin, F., Chu, W., Tao, Y., Majeed, N., Hudson, T., Narod, S. A., and Pollak, M. Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. Cancer Epidemiol. Biomark. Prev., *10:* 377–384, 2001.

23. Lamberts, S. W., van den Beld, A. W., and van der Lely, A. J. The endocrinology of aging. Science (Wash. DC), *278:* 419–424, 1997.

24. Janssen, J. A., Stolk, R. P., Pols, H. A., Grobbee, D. E., de Jong, F. H., and Lamberts, S. W. Serum free IGF-I, total IGF-I, IGFBP-1 and IGFBP-3 levels in an elderly population: relation to age and sex steroid levels. Clin. Endocrinol., *48:* 471–478, 1998.

25. Fazio, S., Sabatini, D., Capaldo, B., Vigorito, C., Giordano, A., Guida, R., Pardo, F., Biondi, B., and Sacca, L. A preliminary study of growth hormone in the treatment of dilated cardiomyopathy. N. Engl. J. Med., *334:* 809–814, 1996.

26. Landin-Wilhelmsen, K., Wilhelmsen, L., Lappas, G., Rosen, T., Lindstedt, G., Lundberg, P. A., and Bengtsson, B. A. Serum insulin-like growth factor I in a random population sample of men and women: relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. Clin. Endocrinol., *41:* 351–357, 1994.

27. Weissberger, A. J., Ho, K. K., and Lazarus, L. Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-hour growth hormone (GH) secretion, insulin-like growth factor I, and GH-binding protein in postmenopausal women. J. Clin. Endocrinol. Metab., *72:* 374–381, 1991.

28. Goodman-Gruen, D., and Barrett-Connor, E. Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. Am. J. Epidemiol., *145:* 970–976, 1997.

29. Clarke, R. B., Howell, A., and Anderson, E. Type I insulin-like growth factor receptor gene expression in normal human breast tissue treated with oestrogen and progesterone. Br. J. Cancer, *75:* 251–257, 1997.

30. Rubin, M., Fenig, E., Rosenauer, A., Menendez-Botet, C., Achkar, C., Bentel, J. M., Yahalom, J., Mendelsohn, J., and Miller, W. H. J. 9-*cis* Retinoic acid inhibits growth of breast cancer cells and down-regulates estrogen receptor RNA and protein. Cancer Res., *54:* 6549–6556, 1994.

31. Roman, S. D., Ormandy, C. J., Manning, D. L., Blamey, R. W., Nicholson, R. I., Sutherland, R. L., and Clarke, C. L. Estradiol induction of retinoic acid receptors in human breast cancer cells. Cancer Res., *53:* 5940–5945, 1993.

32. Demirpence, E., Balaguer, P., Trousse, F., Nicolas, J. C., Pons, M., and Gagne, D. Antiestrogenic effects of all-*trans*-retinoic acid and 1,25-dihydroxyvitamin D_3 in breast cancer cells occur at the estrogen response element level but through different molecular mechanisms. Cancer Res., *54:* 1458–1464, 1994.

33. Chambon, P. A decade of molecular biology of retinoic acid receptors. FASEB J., *10:* 940–954, 1996.

34. Cobleigh, M. A., Gray, R., Graham, M., Norton, L., Martino, S., Budd, G. T., Ingle, J. N., Davidson, N. E., and Wood, W. C. Fenretinide (FEN) *vs* placebo in postmenopausal breast cancer patients receiving adjuvant tamoxifen (TAM), an Eastern Cooperative Oncology Group Phase III intergroup trial (EB193, INT-0151). Proc. Am. Soc. Clin. Oncol., *19:* 86a, 2000.

35. Torrisi, R., Mezzetti, M., Johansson, H., Barreca, A., Pigatto, F., Robertson, C., and Decensi, A. Time course of fenretinide-induced modulation of circulating insulin-like growth factor (IGF)-i, IGF-II and IGFBP-3 in a bladder cancer chemoprevention trial. Int. J. Cancer, *87:* 601–605, 2000.

36. Deal, C., Ma, J., Wilkin, F., Paquette, J., Rozen, F., Ge, B., Hudson, T., Stampfer, M., and Pollak, M. Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. J. Clin. Endocrinol. Metab., *86:* 1274–1280, 2001.

37. Reed, M. J., Christodoulides, A., Koistinen, R., Seppala, M., Teale, J. D., and Ghilchik, M. W. The effect of endocrine therapy with medroxyprogesterone acetate, 4-hydroxyandrostenedione or tamoxifen on plasma concentrations of insulin-like growth factor (IGF)-I, IGF-II and IGFBP-1 in women with advanced breast cancer. Int. J. Cancer, *52:* 208–212, 1992.

38. Singer, C., Rasmussen, A., Smith, H. S., Lippman, M. E., Lynch, H. T., and Cullen, K. J. Malignant breast epithelium selects for insulin-like growth factor II expression in breast stroma: evidence for paracrine function. Cancer Res., *55:* 2448–2454, 1995.

39. Lonning, P. E., Lien, E. A., Lundgren, S., and Kvinnsland, S. Clinical pharmacokinetics of endocrine agents used in advanced breast cancer. Clin. Pharmacokinet., *22:* 327–358, 1992.

40. Yee, D., Chavez, J. B., Ruan, W. F., and Kleinberg, D. L. Inhibition of normal mammary gland development and breast cancer growth by insulin-like growth factor binding protein. Proc. Am. Soc. Clin. Oncol., *19:* 209a, 2000.

41. Kaaks, R., Toniolo, P., Akhmedkhanov, A., Lukanova, A., Biessy, C., Dechaud, H., Rinaldi, S., Zeleniuch-Jacquotte, A., Shore, R. E., and Riboli, E. Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J. Natl. Cancer Inst., *92:* 1592–1600, 2000.

42. Figueroa, J. A., and Yee, D. The insulin-like growth factor binding proteins (IGFBPs) in human breast cancer. Breast Cancer Res. Treat., *22:* 81–90, 1992.

Long-Term Effects of Fenretinide, a Retinoic Acid Derivative, on the Insulin-like Growth Factor System in Women with Early Breast Cancer

Andrea Decensi, Harriet Johansson, Rosalba Miceli, et al.

Cancer Epidemiol Biomarkers Prev 2001;10:1047-1053.