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[1]

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Abstract

Circulating levels of insulin-like growth factor-I (IGF-I) and insulin-like growth factor-binding protein 3 (IGFBP-3) vary considerably between normal individuals. Recent epidemiological studies have provided evidence that these levels are predictive of risk of several common cancers. To evaluate possible sources of variation of the levels of circulating IGF-I and IGFBP-3 in females, we studied specific candidate genetic and nongenetic factors in 311 nulliparous, premenopausal Caucasian women, 17–35 years of age. Women who used oral contraceptives (OC) had reduced levels of IGF-I (269 *versus* 301 ng/ml; $P = 0.001$ adjusted for age) and increased levels of IGFBP-3 (4213 *versus* 4009 ng/ml; $P = 0.002$, adjusted for age) compared with nonusers. The ratio of IGF-I:IGFBP-3 was associated with the dose of estrogen contained in the OC ($P_{trend} = 0.006$, adjusted for age). We identified a novel single bp polymorphism in the promoter region of the gene encoding IGFBP-3. This polymorphism was related to the level of IGFBP-3 in the circulation. Mean IGFBP-3 levels were 4390, 4130, and 3840 ng/ml for the *AA*, *AC*, and *CC* genotypes, respectively ($P_{trend} = 0.006$, adjusted for age and OC use). We observed no effect of a recently described polymorphism in the promoter region of the gene encoding IGF-I on the plasma IGF-I level, but there was evidence for a modifying effect of this locus on the influence of OC on the IGF-I level. Our results support the view that circulating IGF-I levels and IGFBP-3 levels are complex traits and are influenced by a number of interacting genetic and nongenetic factors.

Introduction

The IGF[3] system plays key roles in regulating cell proliferation and apoptosis, both at whole-organism and cellular levels (1). IGFs have characteristics of both classic endocrine hormones and tissue growth factors. Circulating IGF-I and IGFBP-3 levels are largely dependent on hepatic production and are tightly regulated. In addition, autocrine and paracrine production of IGF-I, IGFBP-3, and other molecules of the IGF system within IGF-responsive tissues are of major physiological importance (1, 2).

There is considerable interindividual variability in circulating levels of IGF-I and IGFBP-3 and possibly parallel interindividual variability in the tissue expression of the genes encoding these proteins. Circulating concentrations of these two proteins are highly correlated because IGFBP-3 carries at least 90% of IGF-I present in the circulation (3–5). However, there are complex equilibria in the circulation whereby IGF-I as well as IGF-II may be present in complexes with IGFBP-3, with other IGF-binding proteins, or in a free state.

Recent epidemiological studies suggest that circulating IGF-I levels are positively correlated with risk of several common cancers and that this risk is attenuated by higher IGFBP-3 levels (*e.g.,* Refs. 6–9). A hypothesis to explain this observation is that the circulating IGF-I:IGFBP-3 ratio represents a surrogate for tissue IGF bioactivity, which is itself a surrogate for (or even a determinant of) cell renewal rate in epithelial cell populations (10). There is evidence that if factors such as mutation rate and DNA repair are held constant, then accumulation of somatic cell genetic damage leading to carcinogenesis will be influenced by kinetics of cell renewal, including factors such as cell turnover rate and probability of survival of partially transformed cells (11, 12).

Twin studies (13, 14) have shown that about 50% of the interindividual variability in circulating IGF-I and IGFBP-3 levels is genetically determined, but specific loci involved have not been described. We undertook studies to evaluate interindividual variability in circulating IGF-I and IGFBP-3 levels in relation to specific genetic and nongenetic factors.

Materials and Methods

Study Subjects

Five hundred and seventeen women between 17 and 35 years of age were recruited from the University of Toronto, from the

[3] The abbreviations used are: IGF, insulin-like growth factor; IGFBP-3, insulin-like growth factor-binding protein-3; BMI, body mass index; OC, oral contraceptive.
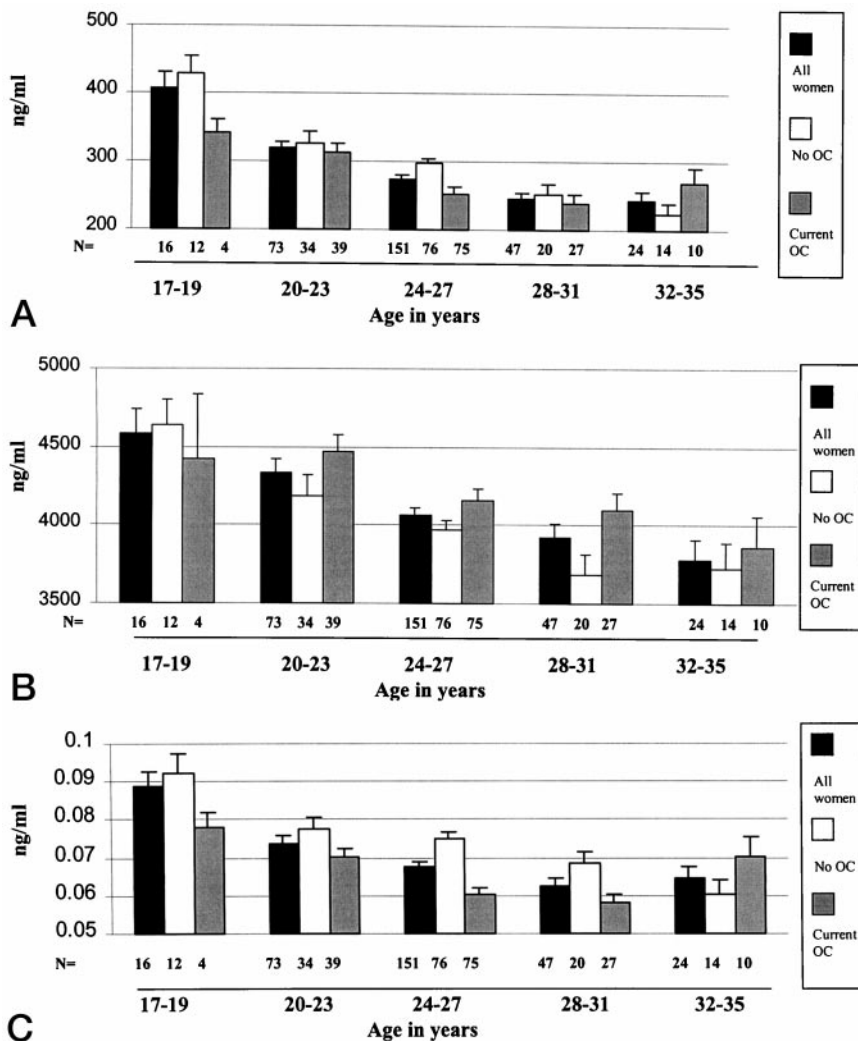
Fig. 1. Effect of age and OC use on plasma IGF-I, IGFBP-3 plasma levels, and their ratio. *A*, Mean plasma IGF-I levels by age; *B*, mean plasma IGFBP-3 levels by age; *C*, mean IGF-I: IGFBP-3 ratio in plasma by age. Results are shown as values $\pm$ SE. IGF-I levels decline with age among nonusers ($R_s = -0.44$; $P = 10^{-6}$) and OC users ($R_s = -0.35$; $P = 6 \times 10^{-6}$). IGFBP-3 plasma levels decline with age among nonusers ($R_s = -0.30$; $P = 0.0001$) and OC users ($R_s = -0.25$; $= 0.002$). Overall, OC users had lower IGF-I levels than nonusers ($\beta = -28.3$; $P = 0.001$, adjusted for age) and higher IGFBP-3 levels than nonusers ($\beta = 226.5$; $P = 0.002$, adjusted for age). The IGF-I:IGFBP-3 ratio declines with age among nonusers ($R_s = -0.34$; $P = 10^{-5}$) and current OC users ($R_s = -0.25$; $P = 0.002$). Overall, OC users had lower IGF-I:IGFBP-3 ratio than nonusers ($\beta = -1.1 \times 10^{-2}$; $P = 5 \times 10^{-10}$, adjusted for age).

Bay Center for Birth Control, and from the Toronto community through flyers and e-mail. The protocol was approved by the Ethics Committee of the University of Toronto. The present study deals with the results on 311 white women. These women had never been pregnant, had no past diagnosis of cancer, and did not have diabetes. The remaining 206 women were excluded for the following reasons; 172 were of other ethnic backgrounds (non-Caucasian), Asian ($n = 77$), Indian-Pakistani ($n = 28$), black ($n = 15$), or other or mixed ethnicity ($n = 52$). Four women had been pregnant, three women had diabetes, one woman had undergone hysterectomy, and one woman was currently using Norplant. Twenty-five women were excluded because of missing information, and six women had missing IGF-I and IGFBP-3 values. We were unable to reach one woman who had left all of the OC questions blank and 18 women who had not answered the diabetes question.

The mean age of the 311 women included in the study was 25.4 years; 71% had graduated with a degree or diploma from college, 87% were born in Canada, 14% kept a vegetarian diet, 15% were current smokers, and 90% sometimes used alcoholic beverages. The mean age of the 206 women excluded from the study was 25.4 years; 61% had graduated with a degree or diploma, 52% were born in Canada, 10% kept a vegetarian diet,

19% were current smokers, and 73% sometimes used alcoholic beverages. BMI was missing for 16 women who were included in the study. The study protocol included a morning visit to the study center on a random day of the menstrual cycle. All of the women completed a self-administered questionnaire, detailing information on age, ethnic group, menstrual history, current diet (vegetarian or not), exercise, and smoking. A brief medical history included the current use of OCs and other medications and a family history of cancer. Samples of various packages of OC brands were displayed at the center, and the women were asked to identify their current brand. All of the contraceptive formulations contained 17α ethinyl estradiol as estrogen, but the type of progesterone varied. Each type of contraceptive was coded as to the amount of estrogen and the type of progesterone it contained. The average daily ethinyl estradiol dose was calculated for women who used triphasic pills. Seven different types of progesterone were recorded. Women were asked to indicate the date of the first menstrual flow of the previous cycle and were required to report by telephone when the onset of menses began for the next cycle. The date of blood draw was then classified according to the calculated day of the menstrual cycle. Menstrual cycle phase was missing for 39 women, 12 women did not call back with the date of their next period, and
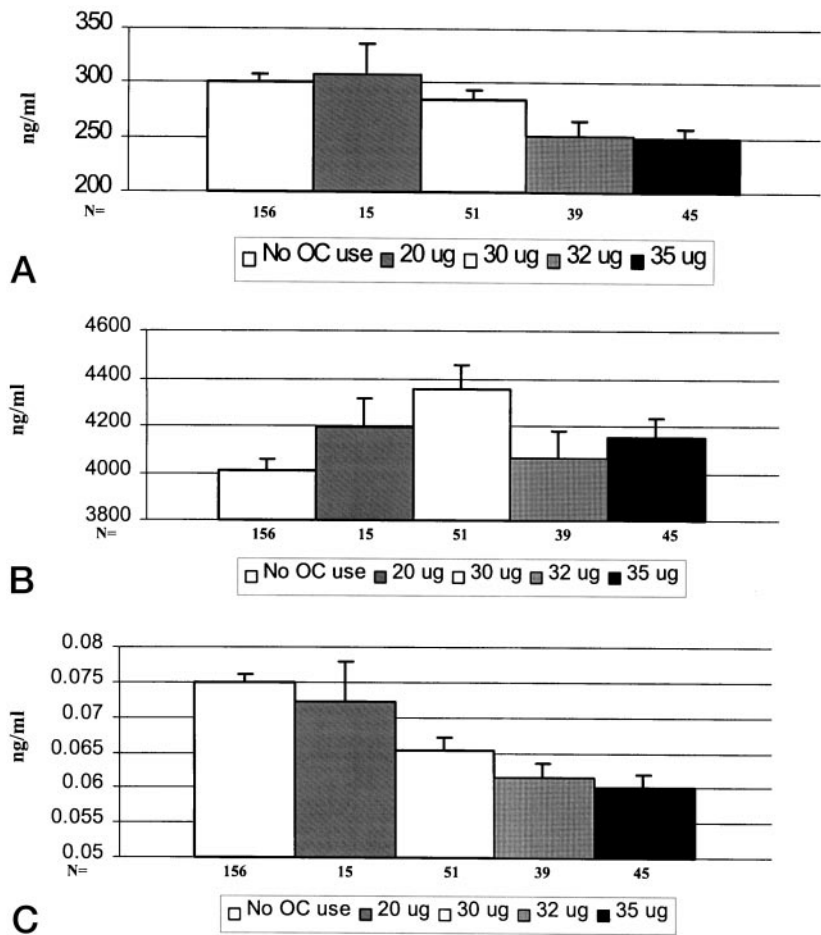
Fig. 2. Effect of oral 17α-ethinyl estradiol dose on plasma IGF-I, IGFBP-3, and their ratio. *A*, mean plasma IGF-I levels by average daily dose of 17α-ethinyl estradiol; *B*, mean plasma IGFBP-3 levels by average daily dose of 17α-ethinyl estradiol; *C*, mean IGF-I:IGFBP-3 ratio in plasma by average daily dose of 17α-ethinyl estradiol. Results are shown as values ± SE. Mean IGF-I levels significantly declined with dose ($\beta = -3.5$; test for trend $P = 0.02$, adjusted for age). Mean IGFBP-3 levels were not related to 17α-ethinyl estradiol dose in current OC users. The mean IGF-I:IGFBP-3 ratio significantly declined with average 17α-ethinyl estradiol dose in current OC users ($\beta = -7.6 \times 10^{-4}$; test for trend $P = 0.006$, adjusted for age).

27 women had irregular periods. Menstrual cycle phase was categorized into seven categories relative to the onset of bleeding and the onset of the next menstrual period. Blood samples were taken for analysis of plasma levels of IGF-I and IGFBP-3 and for DNA extraction.

IGF-I and IGFBP-3 Plasma Levels

Levels of IGF-I and IGFBP-3 in plasma were assayed using ELISA methodology as described previously (7).

Genotyping

IGF1 Polymorphism. Genomic DNA was extracted from 20 ml of peripheral blood using Puregene DNA extraction kits. The previously described variable number tandem repeat polymorphism in the *IGF1* gene (15) was evaluated using the following PCR primers: sense 5′ GCT AGC CAG CTG GTG TTA TT 3′ and antisense 5′ ACC ACT CTG GGA GAA GGG TA 3′. PCR reactions were performed using 25 ng of template DNA for each reaction, 0.4 mM of each primer, 200 mM of each deoxynucleotide triphosphate, 1.0 mM $MgCl_2$, 2% DMSO, 1.25 units of Taq polymerase (Life Technologies, Inc.), and the manufacturer's standard buffers in a final reaction volume of 25 µl. To evaluate the variable number tandem repeat in the *IGF1* gene, the amplified PCR products, labeled with a Cy5 fluorescent label, were subject to acrylamide gel electrophoresis. The gels were dried and scanned on a Storm 860 Phosphorimaging System (Molecular Dynamics, Sunnyvale, CA) to visualize and score the repeat alleles. The number of repeat elements was determined by measuring the size of the PCR products compared with that predicted from the controls.

IGFBP-3 Polymorphism. We identified novel polymorphisms in the IGFBP-3 promoter regions by directed sequencing of 40 genomic DNA samples from a multiethnic Montreal population. A single bp polymorphism at position -202 relative to the CAP site was selected for further study because of preliminary evidence that genotype at this locus was related to circulating IGFBP-3 level. We designed an RFLP assay for this polymorphism. A 50 to 250-ng aliquot of genomic DNA was mixed with PCR buffer, supplemented by 1.6 µM of primers, 1 mM $MgCl_2$, 0.1 mM of each deoxynucleotide triphosphate, 2% DMSO, and 2 units of Taq DNA polymerase (Life Technologies, Inc., Burlington, ON). Primer sense and antisense were 5′-CCA CGA GGT ACA CAC GAA TG-3′ and 5′-AGC CGC AGT GCT CGC ATC TGG-3′, respectively. The cycling parameters consisted of an initial incubation of 10 min at 94°C followed by 35 cycles of 30 s at 96°C, 30 s at 64°C, and 1 min at 72°C. The reaction was terminated after a final extension time of 5 min at 72°C. PCR product (20 µl) was digested with 5 units of *Alw21I* (MBI Fermentas, Flamborough, ON) from 3 to 14 h at 37°C. Digestion products were visualized on a 2% agarose gel stained with ethidium bromide.
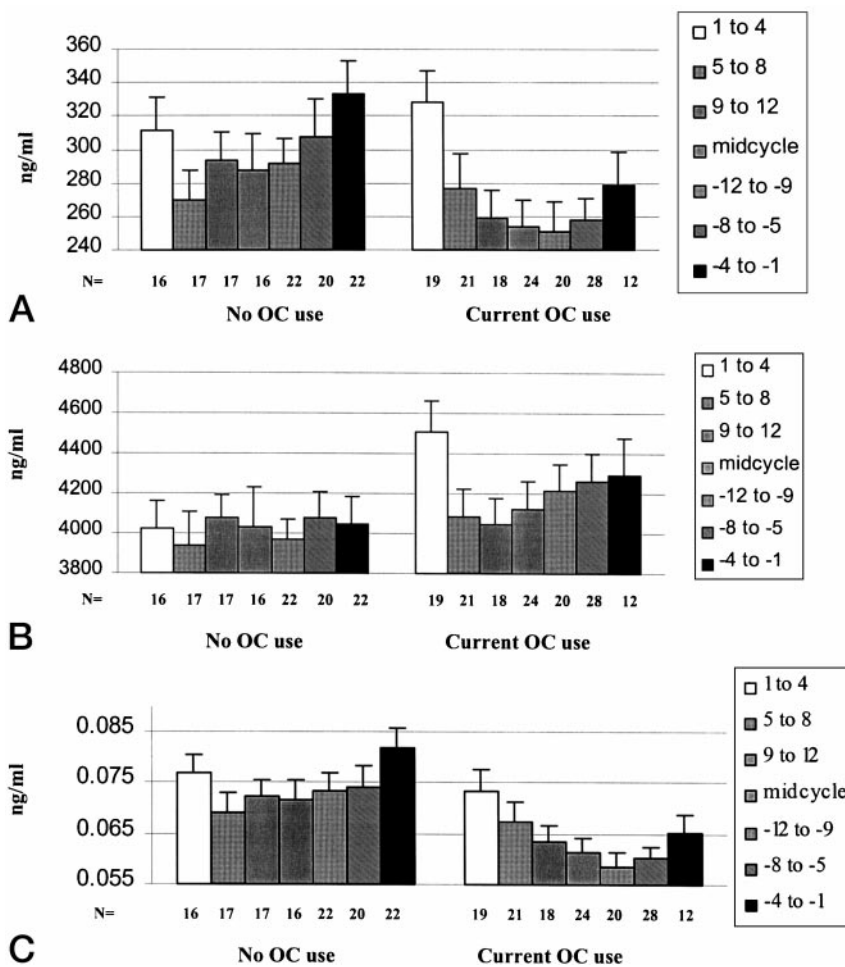
Fig. 3. Relationship of menstrual cycle to plasma IGF-I and IGFBP-3 levels and their ratio. *A*, mean plasma IGF-I levels in relation to day of menstrual cycle; *B*, mean plasma IGFBP-3 levels in relation to day of menstrual cycle; *C*, mean IGF-I:IGFBP-3 ratio in plasma in relation to day of menstrual cycle. Results are shown as values ± SE. Statistical modeling indicated that the observed IGF-I data better fit a parabolic pattern than a straight line (P = 0.002). The IGF-I:IGFBP-3 ratio showed a similar pattern.

Data Analysis

Spearman's correlation coefficient was used to estimate the correlation between levels of IGF-I, IGFBP-3, and age. The effects of other variables were measured using multivariate linear regression models, adjusting for age. Contraceptive preparations were classified according to the dose of estrogen (continuous) and the type of progestin (categorical). The unstandardized regression coefficient was labeled as "β" and presented when a multivariate model was used.

Results

Age. All of the women were between 17 and 35 years of age. The mean plasma IGF-I level and the mean plasma IGFBP-3 level declined continuously with age (IGF-I, r_s = −0.40, P = 10^{-6}; IGFBP-3, r_s = −0.27, P = 10^{-6}; Fig. 1). The IGF-I: IGFBP-3 ratio was also negatively associated with age (r_s = −0.30; P = 10^{-7}). The correlation between the IGF-I level and the IGFBP-3 level was 0.561 (P = 10^{-7}).
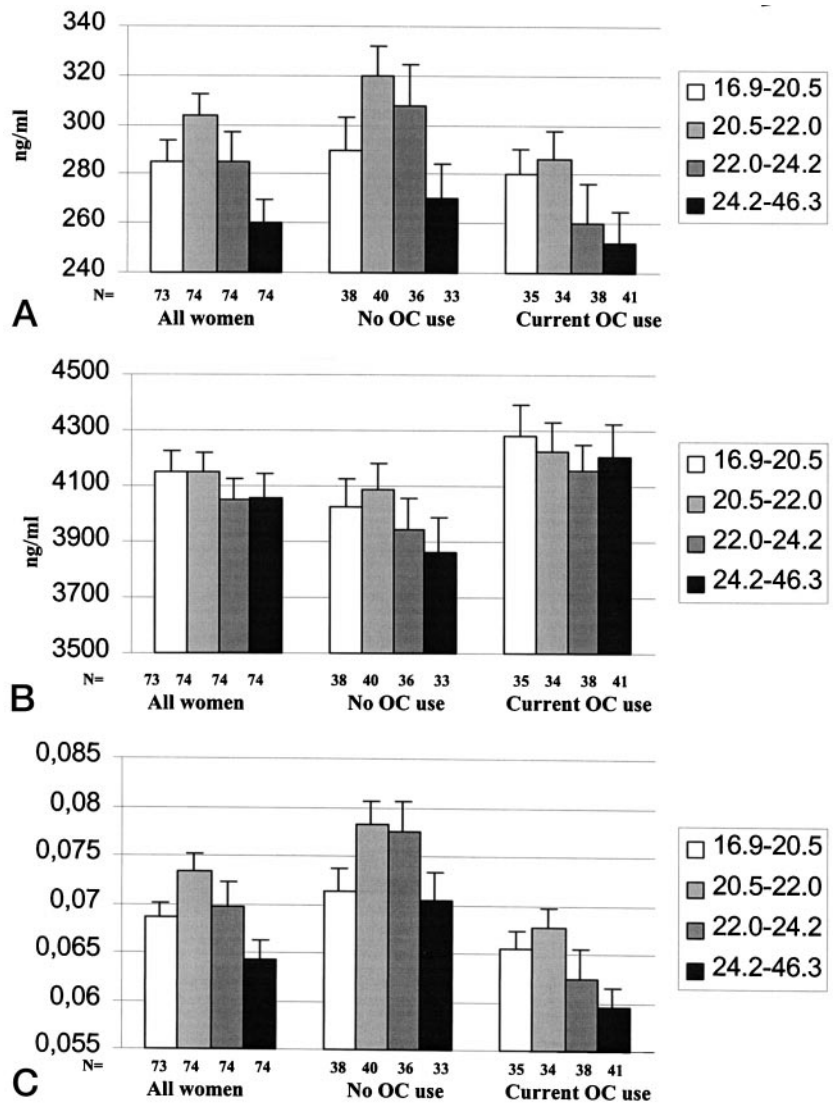
OCs. OC use was an important determinant of the IGF-I level. One hundred and fifty five women (50% of total) were current users of OCs. Women who used OCs had significantly lower IGF-I levels (269 ng/ml *versus* 301 ng/ml; β = −32.3; P = 0.001), significantly higher IGFBP-3 levels (4209 ng/ml *versus* 4013 ng/ml; β = 203; P = 0.006), and significantly lower IGF-I:IGFBP-3 ratios (0.064 *versus* 0.075; β = −1.1 × 10^{-2};

P = 5 × 10^{-10}), compared with nonusers. There were no significant differences between OC users and nonusers in terms of age, age at menarche, height, weight, or BMI. The strength and statistical significance of the association between OC use and IGF-I and IGFBP-3 levels remained essentially the same after adjustment for age.

A clear dose-response relationship was present between the average daily dose of ethinyl estradiol and the plasma IGF-I level (β = −3.52; P = 0.02, adjusted for age; Fig. 2). In contrast, IGFBP-3 levels were not significantly influenced by the estrogen dose. The ethinyl estradiol content was also associated with the IGF-I:IGFBP-3 ratio (β = −8 × 10^{-4}; P = 0.006, adjusted for age). There was no association observed between the IGF-I or IGFBP-3 levels and the type of progesterone used.

Menstrual Cycle. We also evaluated the plasma hormone levels in relation to the day of the menstrual cycle. Among those women who were not taking OCs and who experienced natural menses, the IGF-I:IGFBP-3 ratio was lowest in the early follicular phase, then rose steadily during the follicular and luteal phase, peaking just before menses (Fig. 3). Among OC users, it is common to take the OC for 21 days, followed by 7 days of no pill (or placebo). The IGF-I level declined when the OC was taken and then rose when the placebo was introduced (Fig. 3). The IGF-I:IGFBP-3 ratio showed a similar pattern. Statistical

Fig. 4. Relationship of BMI (kg/m^2) quartile to plasma IGF-I and IGFBP-3 levels and their ratio. *A*, mean plasma IGF-I levels in relation BMI (kg/m^2) quartile; *B*, mean plasma IGFBP-3 levels in relation BMI (km/m^2) quartile; *C*, mean IGF-I:IGFBP-3 ratio in plasma by BMI (kg/m^2) quartile. Results are shown as values $\pm$ SE. There was no significant trend among all of the women or among nonusers. Among current OC users, BMI was negatively associated with the IGF-I plasma level and with the IGF-I:IGFBP-3 ratio ($\beta = 3.7$; $P = 0.04$ and $\beta = 9.1 \times 10^{-4}$; $P = 0.007$, respectively, adjusted for age and estrogen dose).

modeling indicated that the observed IGF-I data better fit a parabolic pattern than a straight line ($P = 0.002$). Among OC users, IGFBP-3 levels were highest during the first 4 days of the menstrual cycle, when placebo pills are consumed. Among nonusers, IGFBP-3 levels showed no consistent pattern over the menstrual cycle.

Age at Menarche. Age at menarche was not significantly associated with IGF-I levels, IGFBP-3 levels, or the IGF-I: IGFBP-3 ratio in the overall study group. However, among OC users, age at menarche was positively associated with the IGF-I plasma levels ($\beta = 10.5$; $P = 0.02$, adjusted for age and estrogen dose) and with the IGF-I:IGFBP-3 ratio ($\beta = 2 \times 10^{-3}$; $P = 0.02$ adjusted for age and estrogen dose). There was no significant association between age at menarche and the IGFBP-3 level.

Height, Weight, and BMI. In the overall study group, there was no significant association between birth weight, height, current weight, or BMI and the plasma level of IGF-I or IGFBP-3. However, in the subgroup of women on OCs, weight was negatively associated with the IGF-I:IGFBP-3 ratio, although the significance level was marginal ($\beta = 2.4 \times 10^{-4}$;

$P = 0.035$, adjusted for age and estrogen dose). In this subgroup, BMI was negatively associated with the IGF-I plasma level and with the IGF-I:IGFBP-3 ratio ($\beta = 3.7$; $P = 0.04$ and $\beta = 9.1 \times 10^{-4}$; $P = 0.007$, respectively, adjusted for age and estrogen dose; Fig. 4).

Smoking, Physical Exercise, and Diet. Neither current nor past smoking, the number of h spent in intense physical exercise/week, a vegetarian diet, nor the amount of coffee consumed were found to be significant predictors of the plasma levels of IGF-I or IGFBP-3 or of the IGF-I:IGFBP-3 ratio. Thirty-one percent of the women had not eaten breakfast at the time of the blood draw. No significant difference in IGF-I or IGFBP-3 plasma levels was seen between women who had eaten breakfast and women who were fasting.

Alcohol. Women who consumed four or more alcoholic drinks/week had significantly lower IGFBP-3 plasma levels than women who drank less (3953 ng/ml *versus* 4173 ng/ml; $\beta = -208$; $P = 0.009$, after adjustment for age and OC use). The suppressive effect of alcohol consumption on IGFBP-3 level was particularly evident in OC users (Fig. 5). IGF-I levels were lower among women who consumed four or more drinks/
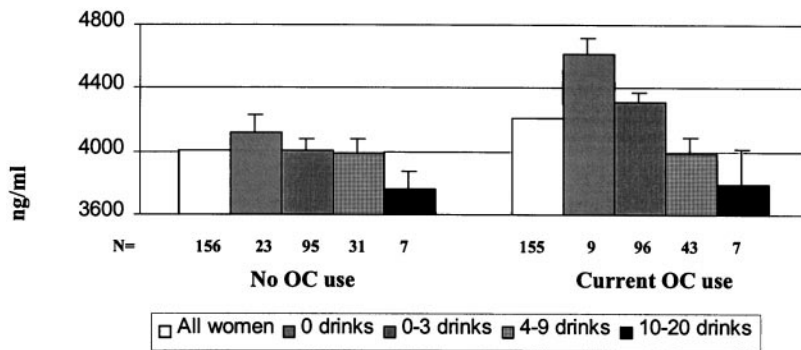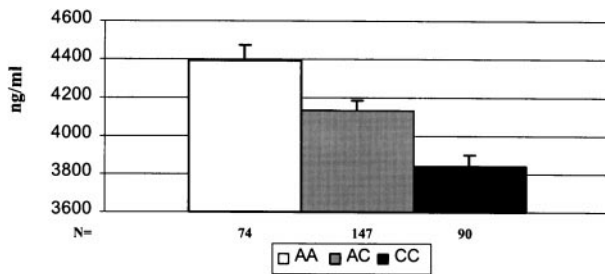
Fig. 6. Mean plasma IGFBP-3 level in relation to the -202 IGFBP3 genotype in all of the women. Results are shown as mean IGFBP-3 plasma levels with ± SE ($\beta = -298$; test for trend $P = 6 \times 10^{-10}$, adjusted for age and current OC use).

Sources of IGF-I and IGFBP-3 Variation. In our population, the circulating level of IGF-I was influenced by age, alcohol use, and OC use. In univariate models, 18% of the variability in the IGF-I level was because of age, 4% was because of OC use, and 2% was because of alcohol consumption. In a multivariate model, these three factors explained 21% of the total IGF-I plasma variation. Overall, the plasma IGFBP-3 levels were significantly influenced by age, by the *IGFBP3* genotype, by alcohol use, and by current OC use. In univariate models, the percentage of variability of IGFBP-3 levels was 9% because of the IGFBP3 polymorphism, 8% because of age, 3% because of alcohol consumption, and 2% because of current OC use. In a multivariate model, 24% of the IGFBP-3 plasma variation was explainable by these four factors.

Discussion

Prior studies concerning circulating IGF-I and IGFBP-3 levels have emphasized issues related to the pathological extremes provided by acromegaly and growth hormone deficiency. Relatively little is known about the determinants of or the clinical significance of the considerable interindividual variability in IGF-I and IGFBP-3 levels within normal populations. However, it is clear that these levels are complex traits determined by interacting genetic and nongenetic factors.

We observed a strong negative correlation between the circulating IGF-I level and age. These results are consistent with previous reports (3–5) and provide evidence that the collection, storage, and assay of our samples were appropriate.

Our findings concerning the influence of OCs confirm and extend prior reports (16) and provide the first evidence for an effect of OCs on IGFBP-3 level. However, caution is required in interpreting the underlying physiology. In menopausal women, transdermal estrogen replacement raises IGF-I levels whereas oral therapy suppresses them (17, 18). Thus, it is possible that the suppressive effects of oral estrogens on serum IGF-I represent a pharmacological effect resulting from hepatic exposure to superphysiological estrogen levels delivered via the portal circulation. It is reasonable to hypothesize that, in general, autocrine and paracrine IGF-I expression in extrahepatic tissues may be regulated in parallel with hepatic expression and circulating level. This assumption may not be valid, however, in the setting of oral estrogen therapy.

The observed variation of IGF-I and IGFBP-3 levels with the menstrual cycle was more marked in the subgroup of women taking OCs. This is not unexpected, because these women have less variability in hormone levels during the menstrual cycle. However, our study is cross-sectional, and it will be necessary to obtain serial measurements in subjects

week, compared with women who drank less (273 ng/ml *versus* 290 ng/ml), but this difference was not significant ($\beta = -8.3$; $P = 0.39$).

Promoter Polymorphism of *IGFBP3*. The -202 polymorphism was a statistically significant predictor of IGFBP-3 plasma levels in young women (Fig. 6). Women with the *AA* genotype had the highest levels of plasma IGFBP-3, women with *AC* were intermediate, and women with *CC* had the lowest IGFBP-3 levels ($\beta = -298$; test for trend $P = 6 \times 10^{-10}$, adjusted for age and OC use). The odds ratio for falling below the median value for IGFBP-3 in the population of women with no variant allele was 2.0 for carriers of one *C* allele ($P = 0.02$) and 5.4 for carriers of two *C* alleles ($P = 6 \times 10^{-6}$). Fig. 7 and Fig. 8 show possible interactions between the *IGFPB3* genotype, the consumption of alcohol, the use of OCs, and plasma hormone levels. Fig. 9 shows the percentage of the IGFBP-3 genotypes by BMI quartile.

Polymorphic Variation of *IGF1*. A recent report by Rosen *et al.* (15) suggested that the number of copies of the common 19-repeat allele in the promoter region of the *IGF1* gene is related to the serum IGF-I level. In our population, plasma levels of IGF-I, IGFBP-3, and the IGF-I:IGFBP-3 ratio were similar among women with zero, one, or two copies of the 19-repeat allele of the *IGF1* gene. However, among the subgroup of women who used OCs, women with at least one copy of the 19-repeat allele had significantly lower IGF-I levels than women without the 19-repeat allele (264 ng/ml *versus* 315 ng/ml; $\beta = -46.3$; $P = 0.025$, adjusted for age and estrogen dose; Fig. 10). The interaction between the presence of the 19-repeat allele of *IGF1* and OC use on the IGF-I level was statistically significant ($\beta = -66.8$; $P = 0.04$). Fig. 11 shows *IGF1* genotype by BMI quartile.

Fig. 7. Mean plasma IGFBP-3 levels in relation to number of alcoholic drinks/week and *IGFBP-3* genotype. Women with a *CC* genotype at the −202 locus of *IGFBP3* not only had the lowest baseline IGFBP-3 level but also showed a trend toward the highest alcohol-induced suppression of levels. Test for trend for alcohol consumption were as follows, adjusted for age and OC use: among all of the women, $\beta = -165$, $P_{\text{trend}} = 0.002$; among women with the *AA* genotype, $\beta = -118$, $P_{\text{trend}} = 0.26$; among women with the *AC* genotype, $\beta = -147$, $P_{\text{trend}} = 0.045$; and among women with the *CC* genotype, $\beta = -181$, $P_{\text{trend}} = 0.03$.
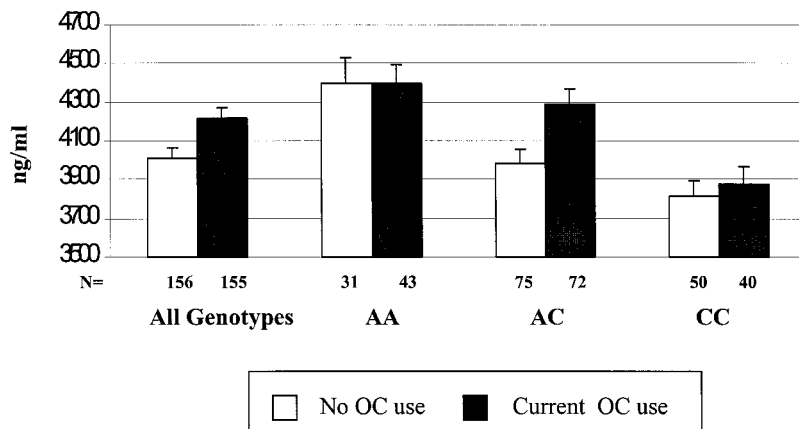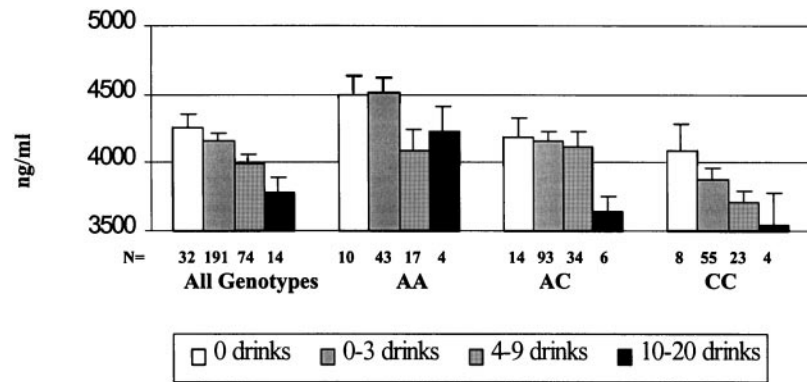

Fig. 8. Mean plasma IGFBP-3 levels in relation to OC use and *IGFBP-3* genotype. Results are shown as mean IGFBP-3 plasma levels with ± SE. None of the significant elevation in IGFBP-3 level associated with OC use seen in population as a whole was related to an effect in women with the *AA* genotype, who had the highest baseline levels. Current OC users with the *AC* genotype had significantly higher IGFBP-3 levels compared with nonusers with the same genotype ($\beta = 355$; $P = 0.0004$, adjusted for age). There was no significant difference between current OC users and nonusers among women with the *CC* genotype after adjustment for age.
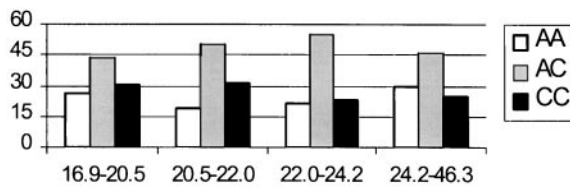

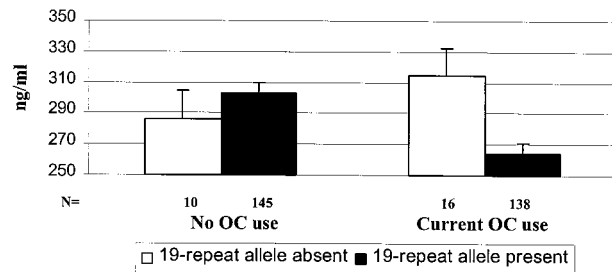Fig. 9. Percentage of women with each *IGFBP-3* genotype by BMI (km/m²) quartile.

Fig. 10. Plasma IGF-I level in relation to OC use and the IGF-I polymorphism described by Rosen *et al.* (15). Results are shown as mean IGF-I plasma levels with ± SE. Overall, there was no effect of the absence or presence of the 19-repeat allele on plasma IGF-I level. There was a significant interaction between absence and presence of the 19-repeat allele and OC use on IGF-I plasma levels ($\beta = -66.8$; $P = 0.04$, adjusted for age, OC use, and *IGF-I* genotype).

throughout the menstrual cycle to obtain precise estimates of the influence of the menstrual cycle on hormone levels. It also will be of interest to determine whether there are subsets of women in whom IGF-I and IGFBP-3 levels vary more or less than the general population with menstrual cycle, inasmuch as prior studies (19, 20) did not detect IGF-I variation with the menstrual cycle. This is an important issue because in clinical practice and in most research studies there is no attempt to control for time of menstrual cycle or exogenous estrogens in interpreting IGF-I levels. We recommend that future epidemiological studies control for these factors in interpreting IGF-I and IGFBP-3 levels.

The −202 *IGFBP3* polymorphism represents a novel genetic factor that has a significant influence on IGFBP-3 plasma levels in young women. *In vitro* functional studies of promoter activity undertaken with the polymorphic variants showed that the *C* allele had 52% of the promoter activity of the *A* allele, in

keeping with the clinical observations reported here.[4] It will be important to determine whether allele frequency at this locus varies with race, because racial variations of IGFBP-3 levels have been reported (21, 22). This may have relevance to cancer risk, either in a direct way or through a modifying effect involving classic environmental or genetic risk factors. For example, there has been speculation that the higher rate of
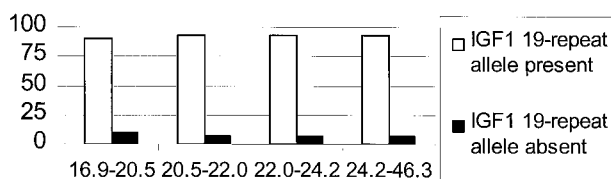
Fig. 11. Percentage of women with each *IGF1* genotype by BMI (km/m^2) quartile.

prostate cancer in African-Americans is related at least in part to their lower IGFBP-3 levels (21, 22). Our study also provides preliminary evidence that genotype at this locus may modify nongenetic factors related to IGFBP-3 level. For example, although in general OC use tends to raise IGFBP-3 level, individuals with an *AA* genotype already have levels higher than the population as a whole, and these are not further raised by OCs.

Our findings with respect to the *IGF1* polymorphism reported by Rosen *et al.* (15) require follow-up because they are based on small subsets. However, the data provide evidence that plasma IGF-I level is paradoxically increased with OC use in the minority of women who lack a 19-repeat allele. If this unusual response to OCs in a genetically defined subset of women is confirmed, it will be important to determine whether there are any clinical correlates. Although most population studies (*e.g.,* Ref. 23) do not demonstrate a substantial increase in breast cancer risk associated with OC use, the possibility that a genetically defined subgroup of women is at increased risk if exposed to OC has not been ruled out.

We hope to extend the present study to identify additional genetic and lifestyle factors that influence plasma levels of IGF-I, IGFBP-3, and other related molecules. The extent to which variability in IGF-I levels and IGFBP-3 levels within the normal range will have clinical relevance, in terms of growth, aging, and cancer risk, is a topic deserving of further study.

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