Lifestyle Correlates of Plasma Insulin-like Growth Factor I and Insulin-like Growth Factor Binding Protein 3 Concentrations[1]

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Abstract

Plasma levels of insulin-like growth factor I (IGF-I) have been associated with risk of a number of cancers and other diseases. We examined the cross-sectional association of plasma IGF-I and IGF-binding protein 3 (IGFBP-3) levels with age, smoking, physical activity, and reproductive and menopausal factors in 1037 healthy women. Adjusted least-square mean hormone levels across categories of lifestyle factors were calculated by linear regression. Age was inversely associated with IGF-I levels. In multivariate analyses, a higher body mass index (BMI) was associated with higher IGFBP-3 levels (BMI <21 *versus* ≥ 29 kg/m^2, 3879 *versus* 4080 ng/ml; *P* for trend = 0.01). Current use of hormone replacement therapy (HRT) was associated with a lower IGF-I, with oral estrogen being associated with the lowest levels (non-use of HRT *versus* oral estrogen + progesterone *versus* oral estrogen: 181 *versus* 143 *versus* 116 ng/ml; *P* for all comparisons ≤ 0.005). Higher parity was also associated with lower levels of IGF-I (no pregnancies *versus* ≥ 4: 212 *versus* 180 ng/ml; *P* for trend = 0.003). We conclude that age and HRT use, particularly oral estrogen alone, were inversely associated with IGF-I levels. BMI was positively associated with IGFBP-3 levels. This is the first report of an inverse association of circulating IGF-I levels with parity. This association may represent one mechanism by which parity exerts its protective effect on some cancers.

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Introduction

IGFs[3] are known mitogens *in vitro* and in animal studies (1–3), and IGF-I is a peptide hormone important in the growth and function of many organs (4). IGF-I and IGFBP-3 levels are complex traits that exhibit considerable interindividual variability and are related to both genetic and nongenetic factors (5). Higher plasma levels of IGF-I have been associated with increased risk of a number of cancers, including prostate cancer (6–8), colon cancer (9–12), lung cancer (13), and premenopausal breast cancer (14–16). The major binding protein is IGFBP-3; low levels of IGFBP-3 have also been associated independently with an increased risk of cancer (9, 15). The biological plausibility of the IGF-cancer link has been reviewed recently (17). Higher IGF-I levels have also been associated with decreased risk of heart disease and osteoporosis (18, 19).

Knowledge of lifestyle factors associated with IGF levels may elucidate disease mechanisms and offer methods of disease prevention. It is reasonably well established that IGF-I levels decline with age (20–25) and with fasting and malnourishment (26). The association of IGF-I levels with potentially modifiable factors such as body weight, smoking, physical activity, and reproductive factors is less well established. Therefore, we examined the cross-sectional association of IGF-I and IGFBP-3 levels with age, smoking, physical activity, and reproductive and menopausal factors in 1037 healthy women enrolled in the NHS.

Materials and Methods

NHS Subjects and Blood Sample Collection. In 1976, the NHS cohort was established when 121,700 female registered nurses from across the United States, 30–55 years of age, answered a mailed questionnaire on risk factors for cancer and cardiovascular disease. Every 2 years since, we have sent follow-up questionnaires to NHS participants. Information on height, age at menarche, and weight at age 18 was assessed at or close to baseline. Information on parity, age at first birth, age at menopause, smoking status, current weight, family history of breast cancer, personal history of benign breast disease, and physical activity were assessed close to the time of blood draw, typically 1990. Physical activity was measured by a validated and reproducible questionnaire (27) in MET-hours/week. BMI was calculated as weight (in kilograms) divided by height (in meters) squared.

Blood samples were collected in 1989 and 1990 from 32,826 NHS participants who were 43–69 years of age at the time, as detailed previously (28). Each woman was sent a kit containing all supplies needed for blood collection, plus a supplemental questionnaire about menopausal status, recent

[3] The abbreviations used are: IGF, insulin-like growth factor; IGFBP-3, IGF binding protein 3; HRT, hormone replacement therapy; BMI, body mass index; NHS, Nurses' Health Study; MET, metabolic equivalent task.

and current postmenopausal hormone use, time since last meal, and time of day of blood sampling. Participants arranged to have their blood drawn and then mailed the whole-blood sample cooled with an enclosed ice-pack via overnight mail. We have documented previously the stability of IGF-I and IGFBP-3 during the period of transport (14). After receipt in our laboratory, samples were centrifuged, divided, and frozen in the vapor phase of liquid nitrogen in freezers (−130°C or colder). This study was approved by the Institutional Review Board of the Brigham and Women's Hospital.

Women included in this analysis were control subjects in a nested case-control study of plasma hormone levels and breast cancer risk (14). For each case with breast cancer, one control was matched by year of birth, time of day blood was drawn, fasting status, month of blood sampling, menopausal status, and use of postmenopausal hormones at the time of blood collection. In addition, 49 women who had reported very low fat intake (<25 percent and <19 percent of energy on the 1986 and 1990 food frequency questionnaires, respectively) were added to increase the range of fat intake. These cutpoints were chosen to maximize the number of women with low fat intakes on both questionnaires, and all women meeting these criteria were included in this analysis. As fat intake decreased in the entire cohort over time, we were able to use a more stringent criteria for the later (1990) food frequency questionnaire. These samples were interspersed with the control samples and were assayed at the same time. Women defined as postmenopausal had no menses for at least 12 months before blood sampling. To increase the number of premenopausal women, additional women were chosen according to the following criteria: 50 years of age or less at time of phlebotomy, premenopausal at time of phlebotomy (still having cycles and not taking HRT), not previously in any case-control data set, had sufficient plasma available, and had answered the 1990 diet questionnaire. Of women fitting these criteria, 400 were chosen randomly, and 88 additional premenopausal women with additional characteristics were oversampled: 25 women with a family history of breast cancer, 25 women reporting ≥15 g of alcohol daily in 1990, and 38 women with a reported fat intake <25% of calories in 1986 and ≤20% in 1990, and ≤2 drinks of alcohol/day and total daily energy intake between 1000 and 3000 kilocalories and BMI between 18 and 30 in both 1986 and 1990. Samples from these women were analyzed in the second batch. Participants had no previously diagnosed cancer (except nonmelanoma skin cancer) and could not have implausible scores for total energy intake (<500 kcal or >3500 kcal/day).

In each case, the factor that was over sampled is either hypothesized or known to affect cancer risk. If IGF-I or IGFBP-3 levels are correlated with these factors, then IGF-I or IGFBP-3 might be the mechanism by which these factors affect cancer risk. However, the distribution of these factors in the NHS population is such that a random sample would not provide enough women in the extremes of the distribution to test these associations, and thus we oversampled women in these extreme categories.

Laboratory Analysis. IGF-I and IGFBP-3 levels for the plasma samples used in this analysis were assayed in two batches in 1996 and 1998. The within-batch laboratory coefficients of variation were <15 and <16% for IGF-I and IGFBP-3, respectively. Details of the assays for IGF-I and IGFBP-3 levels have been reported previously (14). There is evidence that a single plasma IGF-I measurement, such as we have, reflects longer-term circulating IGF-I concentrations; a correlation coefficient of 0.94 was found among 24 adults who had two blood samples drawn ~6 weeks apart (23). IGF-I and IGFBP-3 were assayed by ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX) by a method described previously (14), which is more reproducible and appropriate for large numbers of samples than the RIA used previously (29).

Statistical Analysis. Statistical analyses were done with SAS software (SAS Institute, Cary, NC). The distribution of levels for each hormone was determined. We excluded 3 women whose hormone values were greater than the absolute value of the 75th percentile plus three times the interquartile range. Adjusted mean hormone levels across categories of lifestyle factors were calculated by regressing hormone levels on potential confounders, adding the mean hormone level to the average of the residuals (30). These mean hormone levels are the predicted levels at the reference values of the confounders. The robust variance was used to insure valid inference, even if the regression residuals were not normally distributed (31). *P*s are reported for the linear trend across categories when the underlying variable is a continuous measure, such as age or BMI. For categorical variables (such as smoking history or family history of cancer), the *P* reported is for the difference between the extremes of categories.

Results

There were 1037 women included in the study. In 1990, participants in the study had a median (10th–90th percentile) age of 50.5 (45–66) years and a median BMI of 24.1 (21–34) kg/m^2. Median physical activity was 15 (2–50) MET-hours/week. Forty-seven % had never smoked, 40% were past smokers, and 13% were current smokers. Eight % were nulliparous, 38% had had 1 or 2 children, and 54% had had 3 or more children. Of the parous women, 71% had ever breastfed their infants. Fifty-one % were premenopausal, 44% postmenopausal, and in 5%, menopausal status was not determined. Of those women who were postmenopausal, 67% were not currently using HRT, 16% used oral estrogen and progesterone in combination, 9% used oral estrogen alone, and 8% used other hormone preparations.

The median value for IGF-I was 180 ng/ml, and the tenth percentile to the ninetieth percentile range was 102–289 ng/ml. The comparable median value for IGFBP-3 was 3980 ng/ml (2946–5129).

We evaluated the age-adjusted association of several lifestyle factors with levels of IGF-I and IGFBP-3. Levels of IGF-I and IGFBP-3 did not differ across categories of height, BMI at age 18, waist:hip ratio, personal history of benign breast disease, family history of either breast or colon cancer, age at menarche, age at first birth, menopausal status, or age at menopause. Current users of any type of HRT had lower IGF-I compared with women who had never used HRT. Women who had used HRT in the past (>3 months previously) had mean levels similar to those of never-users (181 and 182 ng/ml, respectively). IGF-I levels were associated with the type of HRT used. Although numbers of women in each category become small, 63 women taking oral estrogen alone had the lowest mean IGF-I level (117 ng/ml), followed by those 56 women taking oral estrogen plus progesterone in combination (142 ng/ml). The 7 women using estrogen in a skin patch form had a mean IGF-I level (190 ng/ml) even higher than women not using HRT (difference not statistically significant). We found decreased levels of both IGF-I and IGBP-3 with increasing duration of use for estrogen plus progesterone (for <5 years *versus* ≥5 years, 161 *versus* 117 ng/ml of IGF-I and 4248 *versus* 3622 ng/ml of IGFBP-3; $P = 0.001$ for both compari-

Table 1 Multivariate adjusted mean levels of IGF-I and IGFBP-3, by category of lifestyle factor

All factors are mutually adjusted for each other plus laboratory batch and menopausal status. *P*s refer to the linear trend test for ordinal variables (age, BMI, physical activity, parity) and a test of difference between extreme categories for nonordinal variables (smoking = never *versus* current; lactation, hormone use = never *versus* oral estrogen only, duration of oral estrogen + progesterone).

	Category definition	IGF-I (ng/ml)	IGFBP-3 (ng/ml)
Age (yr)	<45	218	3974
	45–49	199	3953
	50–54	185	3966
	55–59	177	3973
	60–64	184	4154
		$P = 0.006$	$P = 0.24$
Body Mass Index (kg/m^2)	<21	184	3879
	21–22.9	192	3916
	23–24.9	197	4073
	25–28.9	192	4054
	≥29	181	4080
		$P = 0.66$	$P = 0.01$
Smoking	Never	190	3994
	Past	195	4075
	Current	176	3847
		$P = 0.03$	$P = 0.07$
Physical Activity (MET-hours/week)	<4	184	3961
	4–11.9	186	4064
	12–24.9	195	4020
	≥25	194	3977
		$P = 0.07$	$P = 0.79$
Parity	0	212	4047
	1	190	3939
	2	192	4048
	3	192	4016
	≥4	180	3924
		$P = 0.003$	$P = 0.29$
Lactation[a]	Never	182	3862
	Ever	192	4069
		$P = 0.05$	$P = 0.002$
Hormone use[b]	Never	181	4239
	Current oral estrogen + progesterone	143	3979
	Current oral estrogen only	116	3942
		$P = 0.0001$	$P = 0.02$
Duration of oral estrogen + progesterone[b]	<5 yr	157	4123
	≥5 yr	122	3765
		$P = 0.01$	$P = 0.09$

[a] Parous women only.
[b] Postmenopausal women only.

sons). We did not see an association for duration of use of estrogen alone (for <5 years *versus* ≥5 years, 115 *versus* 119 ng/ml of IGF-I, $P = 0.74$, and 3966 *versus* 3936 ng/ml of IGFBP-3; $P = 0.91$); however, we had limited ability to detect a difference because of the small numbers of women in these categories (14 *versus* 49).

Table 1 includes factors that had statistically significant associations with either IGF-I or IGFBP-3 in the age-adjusted analysis: age, BMI, smoking, physical activity, parity, history of breastfeeding, type of HRT used, and duration of oral estrogen plus progesterone use. All factors were included in a model whereby they were adjusted for each other, as well as laboratory batch and menopausal status. Multivariate results did not differ substantially from age-adjusted results. Age and current smoking were inversely associated with IGF-I levels. Physical activity and ever lactating had a borderline positive association with IGF-I. Women with four or more pregnancies had IGF-I levels that were on average 14% lower than that in nulliparous women. Compared with postmenopausal women not using HRT, women taking oral estrogen alone had 36% lower IGF-I levels, and those taking oral estrogen plus progesterone in combination had 21% lower IGF-I levels. Higher BMI and ever lactating were associated with higher levels of IGFBP-3.

Methods used to estimate the biologically active fraction of IGF-I did not change the results. The molar ratio of IGF-I: IGFBP-3 has been proposed to reflect tissue bioactivity (32). We calculated it as (0.130 × IGF-I concentration in ng/ml) / (0.036 × IGFBP-3 concentration in ng/ml) for each of the factors in Table 1. The results for the molar ratio were consistent with the results in Table 1. Age, parity, and HRT use, which were inversely associated with IGF-I, were also inversely associated with the molar ratio, whereas the inverse associations of smoking and duration of estrogen plus progesterone use with IGF-I were attenuated. Physical activity had a slightly stronger positive association with the molar ratio (lowest *versus* highest category, 0.167 *versus* 0.178; $P = 0.01$) than with IGF-I. BMI, which had a positive association with IGFBP-3, had an inverse association with the molar ratio (lowest *versus* highest category, 0.173 *versus* 0.162; $P = 0.03$). Lactation, which was positively associated with both IGF-I and IGFBP-3, had no association with the molar ratio.

We also calculated mean levels of IGF-I across categories of predictors, controlling for levels of IGFBP-3 (and other covariates) and likewise, levels of IGFBP-3 controlling for levels of IGF-I. Those factors found to be associated with IGF-I or IGFBP-3 in Table 1 were still associated with this additional control, except that the borderline positive association of lactation with IGF-I was now attenuated. In addition, age was now positively associated with IGFBP-3 (youngest *versus* oldest category, 3797 *versus* 4198 ng/ml; $P = 0.003$), and BMI had a borderline inverse association with IGF-I (lowest *versus* highest category, 189 *versus* 178 ng/ml; $P = 0.04$).

In another publication, we show the association of various dietary factors with IGF levels, in particular with total energy and dairy food intake (33). Further adjustment of the factors in Table 1 for energy and dairy food intake did not substantially affect the results shown here.

Discussion

We found associations between lower IGF-I levels and older age, current smoking, lower physical activity, increased parity, lack of breastfeeding, and use of HRT, especially oral estrogen. In addition, both increasing BMI and a history of ever breastfeeding were associated with higher levels of IGFBP-3. The difference in IGF-I levels between nulliparous women and women having four or more births was 32 ng/ml. In this cohort, an increase of 32 ng/ml of IGF-I was associated with a relative risk of 1.24 for premenopausal breast cancer.

The decrease in IGF-I levels with aging has been described many times (20–25, 34). Both the Framingham Heart Study, (22) with 790 men and women, and the Rancho Bernardo Study, (23) with 839 men and women, described a linear decrease in IGF-I levels with age. These latter two studies were the largest to examine various risk factors associated with IGF-I levels before the present study. The inverse association we found between IGF-I and age is consistent with these other studies. The association that we found between IGFBP-3 and age was null when not controlled for IGF-I and positive after

control. This is contrary to the inverse association seen elsewhere (35) but is similar to the relationship found in another group of NHS participants (10).

IGF-I levels reflect growth hormone levels, (36, 37), and people with growth hormone deficiency have decreased muscle mass and increased body fat (38). Because of this, it has been hypothesized that low IGF-I levels are associated with high body fat, weight gain over time, and high BMI (22). A study of 392 Swedish men and women found an inverse association between IGF-I levels and BMI (25). We observed a positive association primarily between IGFBP-3 and BMI, which would lower biologically active IGF-I levels. However, this association was not seen in either the Framingham cohort (22) or in the Rancho Bernardo study (23). The authors of the former study speculate that IGF-I may lose its correlation with body composition in older ages; the mean age in both of these studies was >75 years (22, 23).

The relationship between physical activity and IGF-I levels is complex. Horber *et al.* (39) reported higher IGF-I levels among 14 male joggers with an average age of 67 years than among a similar group of sedentary men. No difference in IGF-I levels was seen among men or women who exercised more or less than 3 h/week in the Rancho Bernardo study (23). No association of IGF-I levels with physical activity was seen in the Framingham study, but physical activity was measured 20 and 36 years before blood sampling and therefore is likely to be misclassified (22). We observed a modest increase in IGF-I levels with increasing current physical activity among the women in our study. This result is not consistent with the apparent protective effect of physical activity on certain cancers (40–42), which presumably occurs through a mechanism unrelated to IGFs.

There have been conflicting reports about the association of smoking with IGF-I levels. A cross-sectional study of 130 Greek subjects found a positive association between IGF-I levels and pack-years of smoking but a negative association between pack-years or the number of cigarettes/day and levels of IGFBP-3 (43). Landin-Wilhelmsen *et al.* (25) reported an inverse association between IGF-I levels and smoking among men and not women. We observed a modest decrease in IGF-I levels associated with current smoking.

We observed decreased levels of IGF-I with higher parity. This association has not been reported previously. This observation is consistent with the lowered risk of breast cancer (44, 45) and colon cancer (46, 47) associated with higher parity. Lower levels of IGF-I could be one mechanism by which parity confers these risk reductions.

IGF-I and IGFBP-3 have been shown to be associated with lactation in animals and humans. Both IGF-I and IGFBP-3 decreased involution of the mammary glands after lactation in mice (48). Normally lactating women who were treated with growth hormone in a randomized trial had elevated plasma concentrations of IGF-I, which were correlated with increased milk volume (49). In addition, long hours of daylight increase levels of IGF-I in lactating cows, independent of growth hormone levels; the increased IGF-I levels preceded and were correlated with increased milk production (50). However, the long-term effect of a history of lactation on levels of IGFs in women is not known. We observed a modest increase in IGF-I and IGFBP-3, with a history of ever breastfeeding. Although we looked for it, we did not see clear-cut evidence of a dose-response with increasing months of breastfeeding.

Previous studies reporting the effect of HRT on plasma IGF-I levels have been somewhat inconsistent, but the majority report decreased IGF-I levels with oral administration of conjugated estrogens. In a retrospective study of 443 Italian women, no difference was seen in IGF-I levels between groups taking no HRT and those taking various combinations of oral and transdermal estrogen and progesterone (51). However, in three small intervention studies of 14–40 women, treatment with oral estrogen caused a decrease in IGF-I levels, whereas treatment with transdermal estrogen did not affect levels (52–54). In addition, in two cross-sectional studies including 39 women and 672 women, respectively, the lowest levels of IGF-I were found in women taking oral estrogen alone, with higher levels among women taking oral estrogen plus progesterone, and with the highest levels among postmenopausal women not currently taking hormones (55, 56). This latter study of 672 women in Rancho Bernardo also found a significant linear decrease in age-adjusted IGF-I levels by duration of use in all current HRT users (56).

Our results were consistent with these last five studies. We found the lowest levels of IGF-I in women taking oral estrogen, followed by women using oral estrogen plus progesterone. Both of these groups had lower levels of IGF-I than women using transdermal estrogen or women with no current hormone use. Because we studied only 7 women using transdermal estrogen, we are unable to tell whether IGF-I levels are truly different between women using transdermal estrogen and those using no hormones. Because estrogen levels do not differ in women taking oral estrogen *versus* transdermal estrogen (52), the difference in IGF-I levels between the two groups appears to be attributable to the route of administration, perhaps through the inhibition of hepatic IGF-I production by oral estrogen. Oral progesterone appears to diminish this inhibition. In addition, we found a decrease in IGF-I level with longer duration of oral estrogen plus progesterone use. Our inability to see a similar effect among women using oral estrogen only may be because such an effect does not exist or because of the small numbers of women in that category.

The influence of HRT on IGF levels is one possible mechanism underlying the consistently observed decreased risk of colon cancer with use of HRT (57–61). However, a seeming inconsistency exists when examining the association of HRT with breast cancer. HRT is associated with lower IGF-I levels but increases risk of breast cancer (62–64). However, there is some evidence that IGF-I levels early in life, rather than those in adulthood, may be particularly important in determining risk (14–16, 65, 66). In addition, we speculate that orally administered estrogens expose the liver to superphysiological estrogens via the portal circulation, and that this suppresses hepatic IGF-I production and therefore reduces circulating IGF-I levels. However, extrahepatic tissues in women receiving oral estrogens are exposed to increased estrogens in a physiological rather than pharmacological range, and this may increase IGF-I expression in certain tissues. Therefore, in woman receiving oral estrogens, circulating IGF-I levels may not serve as a surrogate marker for tissue IGF-I expression or bioactivity. Alternatively, the effect of HRT on IGF levels could serve to dampen the observed HRT/breast cancer relationship.

The primary limitation of this study is its cross-sectional nature; there is no way to know whether factors associated with IGF levels determine those levels, or are in fact determined by them. Because subjects were sampled to increase the range of fat and alcohol intake for another study, the distribution of lifestyle factors in the study population does not necessarily reflect the distribution of these factors in the underlying population. The strengths of this study include its large size and the extensive information on lifestyle factors collected over 25 years.

Our most important new finding was an inverse association of circulating IGF-I levels with increasing parity in healthy women. We also found a modest increase in IGF-I with a history of breastfeeding. A limitation of this study is its cross-sectional nature. These novel findings should be studied further. The parity association is consistent with a protective effect of parity on some cancers and may represent one mechanism by which parity exerts this protective effect.

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