Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 975–981

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Genetic regulation of the variation of circulating insulin-like growth factors and leptin in human pedigrees

Ia Pantsulaia[a,b], Svetlana Trofimov[a], Eugene Kobyliansky[a], Gregory Livshits[a,*]

[a]*Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel*
[b]*Department of Biomedicine, Institute of Medical Biotechnology, Georgian Academy of Sciences, Tbilisi 0159, Georgia*

Received 21 September 2004; accepted 15 February 2005

Abstract

Recent literature has shown that circulating levels of insulin-like growth factor I (IGF-I) and/or IGF binding proteins (IGF-BPs) may be of importance in the risk assessment of several chronic diseases including cancer, cardiovascular disease, diabetes mellitus and so on. The present study examined the extent of genetic and environmental influences on the populational variation of circulating IGF-I and IGF-BP-1 in apparently healthy and ethnically homogeneous white families. The plasma levels of each of the studied biochemical indices were determined by enzyme-linked immunoassay in 563 individuals aged 18 to 80 years. Quantitative genetic analysis showed that the IGF-I variation was appreciably attributable to genetic effects (47.1% ± 9.0%), whereas for IGF-BP-1, only 23.3% ± 7.8% of the interindividual variation was explained by genetic determinants. Common familial environment factors contributed significantly only to IGF-BP-1 variation (23.3% ± 7.8%). In addition, we examined the covariations between these molecules and between them and IGF-BP-3 and leptin that were previously studied in the same sample. The analysis revealed that the pleiotropic genetic effects were significant for 2 pairs of traits, namely for IGF-I and IGF-BP-3, and for IGF-BP-1 and leptin. The bivariate heritability estimates were 0.21 ± 0.04 and 0.15 ± 0.05. The common environmental factors were consistently a significant source of correlation between all pairs (barring IGF-I and leptin) of the studied molecules; they were the sole predictors of correlation between IGF-I and IGF-BP-1, and between IGF-BP-1 and IGF-BP-3. Our results affirm the existence of specific and common genetic pathways that in combination determine a substantial proportion of the circulating variation of these molecules.

1. Introduction

The insulin-like growth factors (IGFs) system plays a crucial role in regulating cell proliferation and apoptosis, both at the whole-organism and cellular levels [1]. Alterations in the balance of IGF axis components in the vessel wall influence cell growth, survival, and migration, as well as extracellular matrix synthesis. Recent literature has shown that circulating levels of IGF-I and/or IGF binding proteins (IGF-BPs) may be of importance in the assessment of risk in several chronic diseases including cancer [2], cardiovascular disease, diabetes mellitus [3], and osteoporosis [4], apart from their involvement in growth abnormalities [5,6].

Circulating concentrations of IGF-I are regulated by the growth hormone (GH), nutritional status, age, and other factors [7]. The stability, availability, and bioactivity of circulating IGFs are regulated by their receptors and binding proteins. The high affinity IGF-BP-1-6 with IGFs has been identified as an agent mediating the effects of cellular growth-suppressing mechanisms and suggested their involvement in IGF-I regulation [1,5,8]. It has been shown that whereas IGF-BP-1 regulates the bioavailability of IGF-I in response to food and exercise, IGF-BP-3 is the predominant store and modulator of IGF-I [8,9].

The IGF axis is also engaged in the regulation of body composition and changes in body fat stores. Moreover, current studies have revealed that in a healthy population, IGF-I and IGF-BP-1 circulating levels are inversely correlated with body mass index (BMI) and leptin [10,11]. Even so, the mechanisms whereby fat mass is signaled to the GH/IGF axis are still poorly understood [12-14]. Leptin plays an important role in the regulation of human food intake, energy expenditure, growth, and sexual maturation. On the other hand, leptin stimulates the production of positive effectors of IGF-I synthesis, as well as antagonizes

* Corresponding author. Tel.: +972 3 6409494; fax: +972 3 640 8287.
E-mail address: gregl@post.tau.ac.il (G. Livshits).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.02.014

the actions of the IGF-I [15]. Although the association of these 2 hormonal systems with anthropometric traits and body composition has been under investigation (eg, Ref. [16]), the physiological interactions between leptin and the GH/IGF-I system remain unclear. For example, in pigs, leptin increased the GH but did not affect circulating insulin, IGF-I thyroxine, or glucose concentrations [15]. Leptin also inhibited the stimulatory effects of IGF-I on estradiol production but did not affect the follicle-stimulating hormone and IGF-induced progesterone production [15]. Moreover, the magnitude and the direction of correlations between leptin and IGFs may be sex and obesity dependent [16,17]. Better knowledge on the effect of leptin levels on IGFs metabolism is needed to clarify the mechanisms underlying leptin and IGF axis interactions.

It is presently claimed that circulating levels of some of the IGF system–related factors and leptin are regulated to a substantial extent by genes [18-21], yet there are practically no publications on the contribution of common genetic sources to the covariation between the above molecules. Therefore, the aims of the present investigation were 2-fold: (1) to determine the contribution of genetic factors to variations of plasma concentrations of IGF-I and IGF-BP-1 in an apparently healthy population; (2) to examine the correlations between the circulating levels of IGF-I, IGF-BP-1, and IGF-BP-3 and leptin, and to evaluate the extent to which any observed correlations were caused by shared genetic and environmental factors. We have previously studied the IGF-BP-3 and leptin in the same sample [22,23], but herein, we focused on their correlations with IGF-I and IGF-BP-1. Our major goal was to ascertain how much of the encountered phenotypic covariation between the selected molecules is attributable to common (pleiotropic) genetic effects, and how much to shared environmental conditions.

2. Materials and methods

2.1. Subjects

The present research was conducted on 563 apparently healthy individuals, 283 men and 280 women, 18 to 80 years old and belonging to 106 nuclear and more complex 3-generation families. All families were of Chuvashian ethnic origin (whites) and have resided for many generations in the Bashkortostan Autonomic Region (Russian Federation). The sampled families were picked at random, without advanced knowledge of the outcome of any of the studied variables. Details on the study design and data collection methods have been published recently elsewhere [24]. Participants in the study had no known chronic or acute infection, hematologic, or other metabolic diseases; they were neither receiving prescription medication or steroidal anti-inflammatory drugs on a regular basis nor consuming vitamin, mineral, or other dietary supplements [24]. All participants were unaware of the specific hypotheses tested out in the study and signed an informed-consent document under the approval of the Ethics Committee of the Tel Aviv University, Tel Aviv, Israel.

2.2. Assays

Blood samples were collected by standard venipuncture technique after a 12-hour fast, and plasma aliquots were stored at −80°C until their use in immunologic analysis. The IGF-I and IGF-BP-1 circulating levels were determined by enzyme-linked immunoassay kits (R&D Systems, Minneapolis, Minn). Protocol for IGF-I included an acid-ethanol extraction to release IGF-I from its binding proteins. Briefly, acid-ethanol mixture (87.5% ethanol/12.5% (vol/vol) hydrochloric acid [2 mol/L]) was added to 200 mL of plasma. After centrifugation at 3000*g* for 30 minutes at 4°C, the supernatant (600 mL) was neutralized with Tris base (0.855 mol/L) and stored at −20°C for 1 hour before a second centrifugation. This latter centrifugation was made at 3000*g* for 30 minutes at 4°C and the supernatants (700 mL) were collected into fresh test tubes. The mean intraassay coefficients of variation were 1.5% and 4.8% for IGF-I and IGF-BP-1, respectively. The mean interassay coefficients of variation for the same array of concentrations were 3.4% for IGF-I and 4.2% for IGF-BP-1. The amount of IGF-BP-3 was measured by double-antibody immunoradiometric assays with reagents from the Diagnostic System Laboratories, Webster, Tex. The leptin plasma levels were measured by enzyme-linked immunoassay using commercial kit (R&D Systems). Additionally measured were the plasma levels of sex hormones. Total testosterone and estradiol values were determined by means of the standard radioimmunoassay (RIA) procedure using TESTO-CT2 and ESTR-US-CT RIA kits (CIS bio international, ORIS Group, France). More detailed information on the sex hormones, IGF-BP-3, and leptin plasma concentration determination has been provided previously [22,23,25].

2.3. Statistical and genetic analysis

Preliminary statistical analyses were performed using the Statistica 6.0 software (Statsoft, Inc, Tulsa, Okla). The distributions of circulating levels of each of the biochemical factors were first examined for possible outliers (http://www.cee.vt.edu/programareas/environmental/teach/smprimer/outlier/outlier.html).

To ensure that some rare observations were not eventually excluded from the analysis, we used 4 SD or more as a criterion. All outliers were reassayed, and only after that, they (3 individuals for IGF-I, 8 individuals for IGF-BP-1, 1 individual for IGF-BP-3, and 5 individuals for leptin) were excluded from further analysis. Because the preliminary analysis revealed significant skewness in the distribution of all the markers, we log transformed all the data before the statistical genetic analysis. However, for the sake of simplicity and for comparison purposes, untransformed data are shown in Table 1. Correlations between plasma levels of IGF-I, IGF-BP-1, IGF-BP-3, and leptin, as well as between them and body height and BMI, were examined first without

Table 1
Descriptive statistics on IGFs and leptin plasma levels in the Chuvashian sample

Variables	Mean ± SD (range)	
	Men (N = 283)	Women (N = 280)
Age (y)	46.36 ± 17.05 (18.00-89.00)	44.61 ± 16.41 (18.00-76.00)
Stature (m)	1.66 ± 0.07 (1.43-1.89)	1.54 ± 0.06 (1.39-1.78)
Weight (kg)	63.93 ± 10.97 (41.10-100.30)	60.79 ± 2.81 (35.50-116.50)
IGF-I (ng/mL)	57.70 ± 22.57 (21.34-150.70)	60.10 ± 26.19 (19.53-201.76)
IGF-BP-1 (ng/mL)	13.04 ± 14.60 (0.58-87.09)	8.26 ± 8.35 (0.37-43.37)
IGF-BP-3 (ng/mL)	2494.92 ± 779.40 (899.10-5212.40)	2873.60 ± 727.89 (1290.90-5228.30)
Leptin (ng/mL)	1.92 ± 1.60 (0.15-9.00)	9.10 ± 7.84 (0.77-55.56)
Testosterone (nmol/L)	15.83 ± 6.74 (0.60-38.70)	1.78 ± 4.38 (0.10-34.90)
Estradiol (pmol/L)	66.16 ± 57.00 (0.10-461.00)	176.48 ± 217.38 (0.70-1492.70)

adjustment for age and in accordance to sex. Next, the contribution of all potential covariates of each of the studied proteins was examined using a multiple regression analysis that takes into account family size and structure. Contribution of the familial effects was evaluated simultaneously with the adjustment. This part of the analysis was conducted using a statistical package FISHER (see succeeding paragraphs). In the female sample, all correlations were first evaluated separately in premenopausal and postmenopausal women. However, because there were no significant differences in the magnitude of correlations between 2 groups of women, we report only correlations computed for the total sample of women.

To examine the genetic architecture of each of the studied traits, we performed variance decomposition analysis using the FISHER statistical package [26], with minor modifications. This method enables to distinguish between different independent components that form the variation of the trait, including additive genetic effect (V_{AD}) and several potential common family environment components (V_{CE}). The latter included variance components attributable to common spouses' environment (V_{SP}), to parent/offspring or household environment (V_{HS}) and to specific shared sibling's environment (V_{SB}). Finally, unexplained residual variation of the trait was defined as V_{RS}. Therefore, the total phenotypic variance was $V_{PH} = V_{AD} + V_{CE} + V_{RS}$. The general model also included regression parameters that estimated the effect of age and other potential covariates on each dependent variable (eg, IGF-I) in men and women separately. A maximum likelihood ratio test was used for comparing between the general model and a more limited model, containing one or more parameters constrained to the expected value. Division of each of the significant components of variation by the total variance gives the standardized components of the variance. For example, the narrow sense heritability ($h^2 = V_{AD}/V_{PH}$) can be defined as the proportion of overall phenotypic variation attributable to additive genetic factors.

To determine the extent of genetic and environmental correlations between the studied variables, a bivariate analysis was undertaken with the same FISHER package. The program computes the variance components for each pair of the traits and also evaluates an additive genetic (r_G) and environmental (r_E) correlation between them [27]. A correlation between genetic factors (r_G) and/or between environmental factors (r_E) suggests that the same genes and/or same environment influence both traits. The statistical significance of r_G and r_E was studied using nested models and examining the change in χ^2 values between the models. In addition, to evaluate the contribution of pleiotropic genetic/familial effects on the variation of each of the biochemical indices, bivariate heritability (h^2_b) was estimated as a parent/offspring cross-correlation between each pair of molecules.

3. Results

3.1. Descriptive statistics

Selected characteristics of the studied population according to sex are shown in Table 1. The IGF-BP-1 levels in average were significantly greater (Mann-Whitney U test, $P < .001$) in men than in women. However, there were no significant differences in IGF-BP-1 levels between pre- and postmenopausal women in this sample ($P > .20$). We do not provide here detailed description of IGF-BP-3 and leptin circulatory levels and their associations with covariates because this was recently reported elsewhere [22,23]. However, their correlations with IGF-I and IGF-BP-1 have not been previously investigated and are therefore reported below.

3.2. Relationships between anthropometric traits and biochemical factors

Exploration of the correlations between the studied dependent variables (leptin, IGF-I, IGF-BP-1, IGF-BP-3) and potential covariates such as age, sex hormones, BMI, and height revealed that IGF-I and IGF-BP-3 declined continuously with age ($r = -0.68$ and -0.56 for IGF-I; $r = -0.27$ and -0.52 for IGF-BP-3, in women and men, respectively, with nominal P values $< .001$). Both IGF-I and IGF-BP-3 showed also modest but consistently significant correlation with body height (ranging between 0.205 and 0.299, with $P < .01$ in all instances). However, in multiple regression analyses, body height made no significant independent contribution to their variation after effect of

Table 2
Phenotypic correlations between circulatory levels of IGFs and leptin in the Chuvashian sample (men—below the empty cells, women—above the empty cells)

	IGF-I	IGF-BP-1	IGF-BP-3	Leptin
IGF-I		−0.19, $P < .001$	0.52, $P < .001$	0.03, $P = .62$
IGF-BP-1	−0.26, $P < .001$		−0.13, $P = .07$	−0.43, $P < .001$
IGF-BP-3	0.60, $P < .001$	−0.19, $P = .01$		0.05, $P = .44$
Leptin	−0.11, $P = .086$	−0.27, $P < .001$	−0.04, $P = .57$	

The data are not adjusted for covariates. For variables' definition, see "Materials and methods" section.

age was taken into account. The correlation between IGF-BP-1 and age was weak and did not achieve statistical significance in either sex (data not shown). Yet, IGF-BP-1 circulatory levels were inversely correlated with BMI (r was between −0.35 and −0.37, $P < .001$) in men and in women, whereas IGF-I and IGF-BP-3 did not correlate with BMI.

There were strong and statistically significant correlations between plasma concentrations of IGF-I and IGF-BP-s in both sexes (Table 2). As expected, the correlation between IGF-I and IGF-BP-3 ($P < .001$, for both men and women) was visibly greater and of the opposite sign from that of IGF-I and IGF-BP-1. The correlation between IGF-BP-3 and IGF-BP-1 levels was also negative, yet it was statistically significant only in men. Finally, highly significant ($P < .001$) negative correlation was observed between IGF-BP-1 and leptin (Table 2).

3.3. Variance decomposition analysis

Table 3 presents the results of analysis of each of the 4 dependent variables separately and shows only parameter estimates for the corresponding most parsimonious models. The implemented models simultaneously evaluated the contribution of potential covariates identified by previous basic statistical examination and that of variance components stemming from different familial factors. Contribution of sex hormones was assessed first separately in pre- and postmenopausal women, and then for the combined female sample. Because significant correlation was found only between estrogen levels and leptin, and only in the female cohort, the estrogen effect was tested only in the models examining leptin variation. Furthermore, because the effects of covariates in preliminary analyses were independent of the menopausal status of the women, Table 3 provides regression coefficients for the entire female sample.

Our statistical genetic analysis clearly revealed a significant and substantial genetic effect on the interindividual variation of each of the biochemical traits. By the likelihood ratio test, the model constraining $V_{AD} = 0$ was rejected for all tested traits ($P < .001$). As seen from Table 3, the additive genetic component explained 47.1% ± 9.0% of the IGF-I circulatory levels variation, whereas for IGF-BP-1, only 23.3% ± 7.8% of the interindividual variation was attributable to genetic determinants. Common familial environment factors contributed significantly only to IGF-BP-1 variation (23.2% ± 7.6%). Genetic effects for IGF-BP-3 and leptin ranged between 47.6% ± 10.4% and 49.2% ± 10%, as already evinced in our previous studies [22,23].

Finally, bivariate genetic analysis was performed to ascertain whether there was a familial basis for the observed phenotypic correlations (Table 4). The parameter estimates obtained in the respective bivariate analyses, with their corresponding standard errors, showed significant contribution of the common environmental sources to correlation

Table 3
Variance decomposition analysis of log-transformed circulating levels of IGFs and leptin in Chuvashian pedigrees

	IGF-I	IGF-BP-1	IGF-BP-3[a]	Leptin[b]
Regression parameter				
α_m			1.301 + 0.174	−0.781 + 0.121
α_f	1.630 + 0.092	−0.019 + 0.064	0.689 + 0.173	−0.781 + 0.121!
β_{tm}	1.630 + 0.092!	−0.034 + 0.064	−0.030 + 0.004	0.019 + 0.002
β_{tf}	−0.036 + 0.002	[0]	−0.016 + 0.004	0.019 + 0.002!
β_{ESTm}	−0.036 + 0.002!	[0]		[0]
β_{ESTf}				0.307 + 0.062
Variance component				
V_{AD}	0.288 ± 0.055	0.265 ± 0.089	0.379 ± 0.083	0.405 ± 0.083
V_{SP}	[0]	[0]	[0]	[0]
V_{HS}	[0]	0.264 ± 0.089	[0]	[0]
V_{SB}	[0]	[0]	[0]	[0]
V_{RS}	0.323 ± 0.044	0.609 ± 0.090	0.418 ± 0.066	0.419 ± 0.066
h^2	47.1% ± 9.0%	23.3% ± 7.8%	47.6% ± 10.4%	49.2% ± 10.1%
c^2	0.0%	23.2% ± 7.8%	0.0%	0.0%

α_m and α_f indicates sex-specific intercepts (m—male, f—female); β_t and β_{EST}, sex-specific regression coefficients for age and circulating estrogen effects. h^2 and c^2 are percentages of the total variance attributable to putative genetic and common environmental effects, respectively; [0], parameter was fixed at the indicated value; !, parameter value was constrained to parameter estimate above. For variables' definition, see "Materials and methods" section.

[a] Data according to Ref. [22].

[b] Data according to Ref. [23].

Table 4
Bivariate statistical genetic analysis of circulating levels of IGFs and leptin. Maximum likelihood parameter estimates with their corresponding standard errors are presented

	IGF-I	IGF-BP-1
IGF-BP-1		
r_G	−0.22 ± 0.16 NS	–
r_E	−0.35 ± 0.08*	–
h_b^2	NS	–
IGF-BP-3		
r_G	0.40 ± 0.14**	0.35 ± 0.223 NS
r_E	0.49 ± 0.09**	−0.33 ± 0.10*
h_b^2	0.21 ± 0.04**	NS
Leptin		
r_G	NS	−0.38 ± 0.16*
r_E	NS	−0.43 ± 0.07**
h_b^2	NS	0.15 ± 0.05**

NS indicates that parameter estimate did not differ significantly from zero; *$P < .05$, **$P < .01$, r_G and r_E estimates of genetic and environmental correlations respectively; h_b^2 indicates bivariate heritability estimate.

between all pairs (but leptin/IGF-I) of the tested biochemical traits. Constraining the corresponding environmental correlations to zero value led to significant deterioration of the model's likelihood (χ_1^2—ranged between 8 and 25 with P values between .004 and <.001). Constraining the respective genetic correlations between IGF-BP-1 and IGF-BP-3, and IGF-BP-1 and leptin to zero did not meaningfully change the maximum likelihood of the corresponding models (P value ranged between .15 and .25). However, pleiotropic genetic effects were significant (with $P < .01$) for 2 other pairs of traits, namely, between IGF-I and IGF-BP-3, and between IGF-BP-1 and leptin (Table 4). The corresponding bivariate heritability estimates were 21.4% ± 4.2% ($P < .01$) and 14.7% ± 5.1% ($P < .05$).

4. Discussion

The present paper assesses putative genetic and common environment influences on IGF-I and IGF-BP-1 variation in a sample of pedigrees randomly collected from an apparently healthy European population. The sample was ethnically homogeneous and therefore our estimates should not be biased or tainted by an admixture of different genetic backgrounds. The present findings indicate that the circulating levels of both IGF-I and IGF-BP-1 do not randomly vary in a healthy population, but rather aggregate in families, where the heritability estimates account for 23.3% (IGF-BP-1) and 47.1% (IGF-I) of the total variation adjusted for covariates such as sex and age (Table 3). The plasma IGF-BP-1 variation appeared to be regulated also by common familial environmental factors (23.2% ± 7.8%). The circulating levels of IGF-I and IGF-BP-1, displayed modest but significant negative phenotypic correlation in both sexes (−0.193 in women and −0.263 in men), which was attributable to environmental factors simultaneously affecting the variation of these 2 molecules (Table 4).

Our results are in good agreement with previously published data, suggesting that some 38% of IGF-I may be attributable to genetic factors, whereas observed genetic contribution to interindividual variation of the IGF-BP-1 levels was much lower, although still statistically significant [19]. The present data are also in accord with the research of Benbassat et al [28] showing not only significant inverse correlation between IGF-I and IGF-BP-1 in elders ($r = -0.40$, $P < .01$), but also demonstrating that this correlation remains significant even after control for insulin and glucose. On the other hand, it is well established that IGF-BP-1 concentrations vary substantially in relation to nutrition, circadian rhythms, and other environmental factors [29,30], which means that the contribution of the genetic component to IGF-BP-1 variation is expected to be modest.

In this context, of special interest are the observations indicating that IGF-BP-1 levels may depend on body mass and plasma leptin [10,31-33]. Our data (Table 2) confirm the cited findings and suggest that the observed phenotypic correlation between IGF-BP-1 and leptin encompasses both pleiotropic genetic effects and shared environmental factors (Table 4). As far as we are aware, this is the first study detecting common genetic and environmental effects on leptin and IGF-BP-1 variation. It is possible that a common household environment for IGF-BP-1, comprising about 23% of its variation, can practically be completely explained in environmental correlation with leptin ($r_E = -0.43$). It is also possible that a substantial portion, if not all of the IGF-BP-1 genetic variation, will be attributed to genetic correlation with leptin ($r_G = 0.38$). On the other hand, estradiol levels significantly influenced leptin's variation in women in our sample. This correlation (Table 3) depended very little on women's menopausal status, once the age differences were taken into account. However, in men, we found no correlation between leptin and either estradiol or testosterone levels. As is known, leptin levels are higher in girls, even before puberty, compared with boys, and independent of differences in body composition [34]. Furthermore, in female rats, ovariectomy caused a decrease in leptin messenger RNA levels in adipose tissue and a decrease in serum leptin concentration, both of which were reversed by administration of estradiol [35]. Thus, our present analysis evinces complicated relationships between different compartments of normal metabolism, with leptin conceivably playing the role of a connecting link between IGF-BP-1 metabolism and estradiol actions [36].

We found no correlation between leptin concentrations and IGF-I and IGF-BP-3, but highly significant ($P < .001$) and substantial correlation (0.52-0.60) was observed between the latter 2 variables in both men and women (Table 2). Such correlation is not unexpected, considering that most of the IGF-I circulating in the blood is attached to

IGF-BPs, whose predominant form in adult human serum is IGF-BP-3 [8,37]. Circulating levels of these 2 molecules vary similarly, increasing during childhood until puberty, and then decreasing with age [29,38], as revealed also in our study. Our findings are also consistent with those of the Framingham Heart Study [39] and the Rancho Bernardo Study [40]. These studies relied on large samples and showed clear linear decrease of IGF-I levels with adult age, similar in extent to what was observed in the Chuvashian sample. The correlation found by us between IGF-I and IGF-BP-3 concentrations is explainable by the fact that the latter carries more than 90% of the IGF-I present in the circulation [41]. Moreover, although various hormonal mechanisms are involved in the regulation of IGF-I and IGF-BP-3, GH is likely the primary regulator of the variation in both these proteins [29,42].

Finally, we may speculate that the present findings suggest that the genetic correlations between examined molecules do not relate to the chromosomal locations of the respective genes. Thus, according to "Online Medical Inheritance in Man" (OMIM), it appears that the IGF-I gene is located on chromosome 12q, whereas both the IGF-BP-1 and IGF-BP-3 genes are mapped to chromosome 7p14-p12. They are close to one another and are arranged in tail-to-tail fashion, separated by 20 kb of DNA. The quantitative trait locus regulating leptin-circulating levels is located on chromosome 2p21. These findings suggest that regulation of the genetic variation and covariation of various metabolites may be dependent not only on their structural genes, but also on other genes. This, however, does not necessarily imply correlation between the genes' location and their interaction. Such an assumption is plausible with our findings of no genetic correlation between the IGF-BP-1 and IGF-BP-3 variations, even though they are located in close linkage, according to OMIM. On the other hand, we found highly significant ($P < .001$) genetic correlations between IGF-BP-1 and leptin, and between IGF-BP-3 and IGF-I (Table 4). Furthermore, we believe that the obtained significant environmental correlations (IGF-I/IGF-BP-1/IGF-BP-3, Table 4) suggest possible Gene × Environment interactions in the regulation of circulating levels of the studied biochemical factors. Clarification of the physiological and genetic mechanisms of these correlations, using modern molecular genetics techniques, is certainly an important and challenging aim for future investigation.

Acknowledgments

This study was supported jointly by grant 1042/04 from the Israel National Science Foundation, by grant 112-551 from Tel Aviv University, Tel Aviv, Israel (Livshits and Kobyliansky), and by a postdoctoral fellowship grant to Pantsulaia, kindly provided by UNESCO and Israel (the Ministry of Education, the Council for Higher Education's Planning and Budgeting Committee, the Ministry of Foreign Affairs, and the Israel National Commission for UNESCO).

References

[1] Renehan A, Zwahlen M, Minder C, O'Dwyer S, Jones J, Clemmons D. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3-34.
[2] Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;9418:1346-53.
[3] Delafontaine P. Insulin-like growth factor I and its binding proteins in the cardiovascular system. Cardiovasc Res 1995;30:825-34.
[4] Sandhu M, Heald A, Gibson J, Cruickshank J, Dunger D, Wareham N. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. Lancet 2002;359:1740-5.
[5] Jehle P, Schulten K, Schulz W, Jehle D, Stracke S, Manfras B. Serum levels of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-1 to -6 and their relationship to bone metabolism in osteoporosis patients. Eur J Intern Med 2003;14:32-8.
[6] Peacey S, Shalet S. Insulin-like growth factor 1 measurement in diagnosis and management of acromegaly. Ann Clin Biochem 2001; 38:297-303.
[7] Gomez JM, Maravall FJ, Gomez N, Navarro MA, Casamitjana R, Soler J. Relationship between 25-(OH) D3, the IGF-I system, leptin, anthropometric and body composition variables in a healthy, randomly selected population. Horm Metab Res 2004;36:48-53.
[8] Nyomba BL, Berard L, Murphy L. Free insulin-like growth factor I (IGF-I) in healthy subjects: relationship with IGF-binding proteins and insulin sensitivity. J Clin Endocrinol Metab 1997;82:2177-81.
[9] Martin J, Baxter R. Inhibition of human fibroblast insulin-like growth factors binding protein (IGFBP) production by IGFBP-3. Endocrinology 1992;131:1568-70.
[10] Soderberg S, Ahren B, Eliasson M, Dinesen B, Brismar K, Olsson T. Circulating IGF binding protein-1 is inversely associated with leptin in non-obese men and obese postmenopausal women. Eur J Endocrinol 2001;144:283-90.
[11] Diéguez C, Carro E, Seoane L, García M, Camina J, Senaris R, et al. Regulation of somatotroph cell function by the adipose tissues. Int J Obes 2000;2:S100-3.
[12] Casanueva FF, Diéguez C. Interaction between body composition, leptin and growth hormone status. Bailliere's Clin Endocrinol Metab 1998;12:297-314.
[13] Gregoire Nyomba B, Johnson M, Berard L, Murphy L. Relationship between serum leptin and the insulin-like growth factor-I system in humans. Metabolism 1999;48:840-4.
[14] Skjaerbaek C, Frystyk J, Kaal A, Laursen T, Moller J, Weeke J, et al. Circadian variation in serum free and total insulin-like growth factor (IGF)-I and IGF-II in untreated and treated acromegaly and growth hormone deficiency. Clin Endocrinol (Oxf) 2000;52:25-33.
[15] Lackey B, Gray S, Henricks D. The insulin-like growth factor (IGF) system and gonadotropin regulation: actions and interactions. Cytokine Growth Factor Rev 1999;10:201-17.
[16] Wolk K, Larsson S, Vessby B, Wolk A, Brismar K. Metabolic, anthropometric, and nutritional factors as predictors of circulating insulin-like growth factor binding protein-1 levels in middle-aged and elderly men. J Clin Endocrinol Metab 2004;89:1879-84.
[17] Seck T, Englaro P, Blum WF, Scheidt-Nave C, Rascher W, Ziegler R, et al. Leptin concentrations in serum from a randomly recruited sample of 50- to 80-year-old men and women: positive association with plasma insulin-like growth factors (IGFs) and IGF-binding protein-3 in lean, but not in obese, individuals. Eur J Endocrinol 1998;138:70-5.
[18] Jernstrom H, Deal Ch, Wilkin F, Chu W, Tao Y, Majeed N, et al. Genetic and nongenetic factors associated with variation of plasma

levels of Insulin-like growth factor-1 and Insulin-like growth factor binding protein-1 in healthy premenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10:377-84.

[19] Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J, Eriksson J, et al. Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. J Clin Invest 1996;98:2612-5.

[20] Rice T, Chagnon Y, Borecki IB, Perusse L, Collier G, Gagnon J, et al. Familial resemblance for plasma leptin: sample homogeneity across adiposity and ethnic groups. Obes Res 2002;5:351-60.

[21] Kaprio J, Eriksson J, Lehtovirta M, Koskenvuo M, Tuomilehto J. Heritability of leptin levels and the shared genetic effects on body mass index and leptin in adult Finnish twins. Int J Obes 2001; 1:132-7.

[22] Pantsulaia I, Trofimov S, Kobyliansky E, Livshits G. Pedigree-based quantitative genetic analysis of interindividual variation in circulating levels of IGFBP-3. J Bone Miner Metab 2002;3:156-63.

[23] Livshits G, Pantsulaia I, Trofimov S, Kobyliansky E. Genetic variation of circulating leptin is involved in genetic variation of hand bone size and geometry. Osteoporos Int 2003;6:476-83.

[24] Livshits G, Karasik D, Kobyliansky E. Complex segregation analysis of the radiographic phalanges bone mineral density and their age-related changes. J Bone Miner Res 2002;17:152-61.

[25] Livshits G, Yakovenko C, Kobyliansky E. Quantitative genetic analysis of circulating levels of biochemical markers of bone formation. Am J Med Genet 2000;94:324-31.

[26] Lange K, Weeks D, Boehnke M. Program for pedigree analysis: MENDEL, FISHER, and dGENE. Genet Epidemiol 1988;5:471-2.

[27] Falconer DS, Mackay TF. Introduction to quantitative genetics, 4th ed. England: Longman; 1996. p. 122-43.

[28] Benbassat CA, Maki K, Unterman T. Circulating levels of insulin-like growth factor (IGF) binding protein-1 and -3 in aging men: relationships to insulin, glucose, IGF, and dehydroepiandrosterone sulfate levels and anthropometric measures. J Clin Endocrinol Metab 1997; 82:1484-91.

[29] Katz L, Rosenfeld R, Cohen P. Clinical significance of insulin-like growth factor binding proteins (IGFBPs). Endocrinologist 1995;5: 36-43.

[30] Wolk A. The growth hormone and insulin-like growth factor I axis, and cancer. Lancet 2004;363:1336-7.

[31] Gómez J, Maravall F, Gómez N, Navarro M, Casamitjana R, Soler J. Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. Clin Endocrinol 2003;58: 213-9.

[32] Lukanova A, Lundin E, Zeleniuch-Jacquott A. Body mass index, circulating levels of sex-steroid hormones, IGF-I and IGF-binding protein-3: a cross-sectional study in healthy women. Eur J Endocrinol 2004;150:161-71.

[33] Mendonca H, Montenegro R, Foss M, Silva de Sa M, Ferriani R. Positive correlation of serum leptin with estradiol levels in patients with polycystic ovary syndrome. Braz J Med Biol Res 2004;37:729-36.

[34] Demerath E, Towne B, Wisemandle W, Blangero J, Chumlea W, Siervogel R. Serum leptin concentration, body composition, and gonadal hormones during puberty. Int J Obes 1999;23:678-85.

[35] Machinal F, Dieudonne M, Leneveu M, Pecquery R, Giudicelli Y. In vivo and in vitro ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. Endocrinology 1999;140:1567-74.

[36] Cardim H, Lopes C, Giannella-Neto D, da Fonseca A, Pinotti J. The insulin-like growth factor-I system and hormone replacement therapy. Fertil Steril 2001;75:282-7.

[37] Rajaram S, Baylink D, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev 1997;18:801-31.

[38] Paolisso G, Ammendola S, Buono A, Gambardella A, Riondino M, Tagliamonte M, et al. Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentrations, insulin action, and cognitive function. J Clin Endocrinol Metab 1997;82:2204-9.

[39] Harris T, Kiel D, Roubenoff R, Langlois J, Hannan M, Havlik R, et al. Association of insulin-like growth factor-I with body composition, weight history, and past health behaviors in the very old: the Framingham Heart Study. J Am Geriatr Soc 1997;45:133-9.

[40] Goodman-Gruen D, Barrett-Connor E. Epidemiology of insulin-like growth factor-I in elderly men and women. Am J Clin Epidemiol 1997; 145:970-6.

[41] Juul A, Hjortskov N, Jepsen L, Nielsen B, Halkjaer-Kristensen J, Vahl N, et al. Growth hormone deficiency and hyperthermia during exercise: a controlled study of sixteen GH-deficient patients. J Clin Endocrinol Metab 1995;80:3335-40.

[42] Tillmann V, Patel L, Gill M, Whatmore A, Price D, Kibirige M, et al. Monitoring serum insulin-like growth factor-I (IGF-I), IGF binding protein-3 (IGFBP-3), IGF-I/IGFBP-3 molar ratio and leptin during growth hormone treatment for disordered growth. Clin Endocrinol 2000;53:329-36.