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Hyperlipidemia is Associated with Altered Levels of Insulin-Like Growth Factor-I

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Summary

Previous studies revealed altered levels of the circulating insulin-like growth factor-I (IGF-I) and of its binding protein-3 (IGFBP-3) in subjects with coronary atherosclerosis, metabolic syndrome and premature atherosclerosis. Hyperlipidemia is a powerful risk factor of atherosclerosis. We expected IGF-I and IGFBP-3 alterations in subjects with moderate/severe hyperlipidemia but without any clinical manifestation of atherosclerosis. Total IGF-I and IGFBP-3 were assessed in 56 patients with mixed hyperlipidemia (MHL; cholesterol >6.0 mmol/l, triglycerides >2.0 mmol/l), in 33 patients with isolated hypercholesterolemia (IHC; cholesterol >6.0 mmol/l, triglycerides <2.0 mmol/l), and in 29 healthy controls (cholesterol<6.0 mmol/l, triglycerides<2.0 mmol/l). The molar ratio of IGF-I/IGFBP-3 was used as a measure of free IGF-I. IHC subjects differed from controls by lower total IGF-I (164±60 vs. 209±73 ng/ml, p=0.01) and IGF-I /IGFBP-3 ratio (0.14±0.05 vs. 0.17±0.04, p=0.04). Compared to controls, MHL subjects had lower total IGF-I (153±54 ng/ml, p=0.0002) and IGFBP-3 (2.8±0.6 mg/ml, p<0.0001), but higher IGF-I/IGFBP-3 ratio (0.25±0.06, p<0.0001). Differences remained significant after the adjustment for clinical and biochemical covariates, except for triglycerides. Patients with both IHC and MHL have lower total IGF-I compared to controls. The mechanism is presumably different in IHC and MHL. Because of prominent reduction of IGFBP-3 in patients with MHL, they have reduced total IGF-I despite the actual elevation IGF-I/IGFBP-3 ratio as a surrogate of free IGF-I.

Key words

Atherosclerosis • Growth factors • Insulin resistance • Cholesterol • Metabolic syndrome

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Introduction

Insulin-like growth factor-I (IGF-I), produced in many tissues, has both local (paracrine) and circulating (systemic) pool, and affects growth and differentiation of cells. It is engaged in the regulation of glucose homeostasis and protein metabolism. Circulating IGF-I originates mainly in the liver and is controlled by pituitary growth hormone (GH). Biological availability of IGF-I is strongly influenced by specific binding proteins (IGFBPs). IGFBP-3 carries more than 80 % of circulating pool of IGF-1 and is also under control of GH (Baxter 1994). IGF-I circulates mainly in the 150 kDa ternary complexes comprising of one molecule each of IGF, IGFBP-3 and the acid-labile subunit (ALS). The latter prolongs IGF-I half-life and prevents its penetration through the endothelium (Boisclair *et al.* 2001).

Serum IGF-I and IGFBP-3 levels decline during adulthood and are involved in physiological changes of aging (Ceda *et al.* 1998). Moreover, circulating IGF-I seems to be an indicator of two leading causes of mortality in western countries: while higher concentrations have been linked to cancers (Ma *et al.* 1999), lower levels were found to be associated with cardiovascular diseases (Spalarossa *et al.* 1996, Schuler-Luttmann *et al.* 2000, Juul *et al.* 2002, Vasan *et al.* 2003, Laughlin *et al.* 2004). The association between IGF-I and atherosclerosis is also supported by experimental research (Tsukahara *et al.* 1994). The reports on the relationship between IGF-I and serum lipids are controversial. Discrepant correlations between total IGF-I and low-

density lipoprotein (LDL) cholesterol were observed in three studies ((Prewitt *et al.* 1992, Ceda *et al.* 1998, Laughlin *et al.* 2004). However, GH replacement therapy (Svensson *et al.* 2002) and IGF-I administration to diabetic subjects (Pratipanawatr *et al.* 2001) resulted in a decline in serum cholesterol and triglycerides. IGF-I and IGFBP-3 have not been studied in subjects with moderate/severe hyperlipidemia, who are at substantial cardiovascular risk. Inclusion of these subjects would help to recognize the relation between lipids and IGF-I/IGFBP system.

Two common hyperlipidemia phenotypes – isolated hypercholesterolemia and mixed hyperlipidemia are associated with different metabolic changes. The latter is usually combined with impaired glucose tolerance (Sandhu *et al.* 2002). We therefore expected that subjects with moderate/severe hyperlipidemia will have reduced total IGF-I and that the type of hyperlipidemia will affect this reduction. Therefore, we compared total IGF-I in patients with isolated hypercholesterolemia (IHC) or mixed hyperlipidemia (MHL) to those in normolipidemic healthy controls. In order to estimate the free fraction of IGF-I, we also analyzed IGFBP-3 in all subjects.

Subjects and Methods

We investigated two groups of hyperlipidemic subjects and a group of healthy controls. Hyperlipidemic subjects were consecutively recruited from patients referred to university hospital based lipid clinic who agreed with the participation in this study. All study subjects were free of clinically manifest vascular disease and did not take any lipid lowering medication for at least 2 months. Patients with secondary hyperlipidemia, alcohol abuse, diabetes mellitus, malignancy, malnutrition or endocrine disorders were excluded. The first group consisted of subjects with mixed hyperlipidemia (MHL group) defined by total cholesterol > 6.0 mmol/l and triglycerides > 2.0 mmol/l. Subject with apo E2/E2 genotype or with lipoprotein lipase deficiency were not included. The second group included subjects with isolated hypercholesterolemia (IHC group) defined by total cholesterol >6.0 mmol/l and triglycerides <2.0 mmol/l. All participants underwent at least two serum lipid analysis prior to the inclusion into this study to ensure correct classification of hyperlipidemic subjects (pre-inclusion values are not shown). The control group consisted of non-obese normolipidemic healthy subjects (total cholesterol <6.0 mmol/l and triglycerides <2.0 mmol/l).

The local Ethical Committee approved the design of the study and all participants signed informed consent. The study conformed to the principles outlined in the Declaration of Helsinki.

Biochemical analyses

The blood samples were drawn in the mornings after 12 h fasting. Serum levels of both IGF-I and IGFBP-3 were measured with an immunoradiometric assay (ImmunoTech, France). Total IGF-I was analyzed after serum extraction with acid ethanol to release IGF-I bound to IGFBPs as described previously (Justová *et al.* 2001). Intra-assay coefficient of variation declared by the manufacturer was 2.9-7.4 % for IGF-I measurement (depending on concentration) and 3.9 % for IGFBP-3. Inter-assay coefficient of variation was 8.9-15.5 % for IGF-I (depending of concentration) and 1.8-3.9 % for IGFBP-3. Serum insulin was also determined by immunoradiometric assay (CIS Bio International, France). Serum total and HDL-cholesterol, triglycerides, and glucose were measured using automated analyzer methods; LDL-cholesterol was calculated according to Friedewald´s formula (data are available only in subjects with triglyceride level below 4.5 mmol/l).

Statistical methods

All variables were expressed as mean ± S.D. with the following two exceptions. Triglyceride and insulin concentrations were logarithmically transformed prior to the analysis in order to normalize their distribution and are expressed as median (range). Molar ratio of IGF-I / IGFBP-3 was used as a measure of free IGF-I. Differences between groups were analyzed by two-tailed t-test for independent samples and by an analysis of covariance (ANCOVA) to adjust for appropriate covariates (age, body mass index - BMI, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and insulin). Inter-related variables (such as total and LDL cholesterol) were included into the analysis separately. $P<0.05$ values were considered as significant.

Univariate correlation analysis was performed separately for each group and then for all subjects together. IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio were correlated with age, BMI, total-, LDL- and HDL-cholesterol, triglycerides, insulin and glycemia. LDL-cholesterol was adjusted only in the comparison of IHC

to controls, because in MHL it was available only in 15 cases. P<0.05 value was considered as significant in between groups analysis. For intra-group correlation analyses, the significance level (p) was lowered to <0.01, and for merged groups analysis to <0.001 to prevent multiple testing bias.

Results

A total of 118 subjects were enrolled: 33, 56, and 29 into IHC, MHL, and control groups, respectively. One outlying non-physiological value of IGF-I in the control group was excluded from the analysis (496 ng/ml) together with IGF-I/IGFBP-3 ratio. These exclusions did not change the significance of performed analyses. Baseline characteristics of studied groups are summarized in Table 1.

Differences in total IGF-I, IGFBP-3, and IGF-I/IGFBP-3 ratio between hyperlipidemic patients and controls are shown in Table 2. Both patient groups had significantly lower total IGF-I compared to controls. This difference was more pronounced in MHL patients. As IHC patients had no change in IGFBP-3, decrease of total IGF-I was associated with significant reduction of free IGF-I fraction. On the other hand, we observed a pronounced decrease of IGFBP-3 in patients with MHL accompanied by a highly significant increase in free IGF-I compared to controls

The differences in IGF-I and IGF-I/IGFBP-3 ratio in IHC compared to controls were adjusted for age, total cholesterol, LDL-cholesterol and triglycerides, i.e. variables that significantly differed in these two groups. Differences of both IGF-I and IGFBP-3 remained significant also after this adjustment.

IGF-I difference between MHC and controls remained significant after the adjustment for age, BMI, total cholesterol, HDL-cholesterol and insulin, but significance disappeared after adding triglycerides. The difference in IGF-I/IGFBP-3 ratio remained significant after the adjustment for age, BMI, total or HDL-cholesterol, but disappeared after adding triglycerides or insulin. IGFBP-3 difference remained significant even after the adjustment for all aforementioned variables.

In IHC group, we observed significant correlation between IGFBP-3 and total cholesterol ($r = 0.47$, $p<0.01$). In MHL group, significant correlations were the following: IGF-I vs. age ($r = –0.52$, $p<0.0001$), IGF-I/IGFBP-3 ratio vs. age ($r = –0.51$, $p<0.01$). In control group, a significant correlation was only for IGF-I vs. age ($r = 0.39$, $p=0.04$). Correlations in merged groups are shown in Table 3.

Table 1. Baseline characteristics of groups.

	Controls (n = 29)	IHC (n = 33)	MHL (n = 56)
Age (years)	47 ± 6	53 ± 10*	49 ± 10
Males/females	10/19	11/22	12/44
BMI (kg/m²)	25.1 ± 3.2	26.6 ± 4.0	28.4 ± 3.7†
Total cholesterol (mmol/l)	5.1 ± 0.2	8.4 ± 1.6‡	7.5 ± 1.5‡
LDL (mmol/l)	3.0 ± 0.6	5.8 ± 1.6‡	4.4 ± 1.1‡
HDL (mmol/l)	1.6 ± 0.3	1.6 ± 0.3	1.2 ± 0.3‡
Triglycerides (mmol/l)	0.9 (2.4)	1.6 (2.6)†	3.8 (17.7)‡
Glycemia (mmol/l)	5.1 ± 0.6	5.2 ± 0.6	5.4 ± 0.7
Insulin (mU/l)	4.5 (37)	4.0 (17.2)	22.2 (68.2)‡

Values shown as mean ± S.D. or median (quartile range). Difference versus controls: * $p<0.05$, † $p<0.001$, ‡ $p<10^{-6}$. IHC: isolated hypercholesterolemia; MHL: mixed hyperlipidemia; BMI: body mass index.

Discussion

We found that hyperlipidemic subjects have significantly lower total IGF-I compared to controls. While in IHC patients the production of IGF-I might really be reduced, in MHL patients, the production of IGF-I is probably increased and low IGFBP-3 is responsible for the total IGF-I reduction. IGF-I/IGFBP-3 ratio was decreased in IHC, but increased in MHL.

The ratio of IGF-I/IGFBP-3 was used as a measure of free IGF-I, similarly as in many other studies (Spalarossa *et al.* 1996, Schuler-Luttmann *et al.* 2000, Juul *et al.* 2002, Vasan *et al.* 2003, Laughlin *et al.* 2004). Free IGF-I could be directly measured by radioimmunoanalysis or by ultrafiltration. Ultrafiltration method is very laborious and technically demanding, while the other method is extremely time-dependent during the incubation, which could easily lead to overestimation of real free IGF-I level. Furthermore, it is practically impossible to separate free IGF-I from that bound to IGFBPs, and this is why some authors argue against direct assessment of free IGF-I. However, it does not fully mirror the complexity of interactions between

Table 2. Differences in IGF-related indices between controls and patients with hyperlipidemia.

	Controls	IHC		MHL	
	mean ± S.D.	mean ± S.D.	P value	mean ± S.D.	P value
Total IGF-I (ng/ml)	196 ± 43	167 ± 63	0.045	152 ± 54	0.0004
IGFBP-3 (mg/ml)	4.3 ± 0.9	4.6 ± 1.1	0.20	2.8 ± 0.9	$<10^{-9}$
IGF-I / IGFBP-3 ratio	0.17 ± 0.04	0.14 ± 0.05	0.03	0.24 ± 0.06	0.00003

IHC: isolated hypercholesterolemia; MHL: mixed hyperlipidemia; IGF-I: insulin-like growth factor-I; IGFBP-3: insulin-like growth factor binding protein-3; IGF-I/IGFBP-3: their molar ratio, which provides a measure of free IGF-I; p (ANCOVA): p value after adjustment for age, gender, body mass index, total cholesterol, and triglycerides.

Table 3. Correlation analysis – merged groups.

	Age	BMI	Total cholesterol	Triglycerides	HDL-cholesterol	LDL-cholesterol	Insulin	Glucose
IGF-I	–0.60*	–0.16	–0.13	–0.08	0.14	0.00	–0.09	–0.23
IGFBP-3	–0.02	–0.10	0.05	–0.42*	0.33	0.25	–0.57*	–0.14
IGF-I/IGFBP-3 ratio	–0.41*	0.06	–0.03	0.37*	–0.36	–0.19	0.63*	–0.03

Subjects from all 3 groups were merged together for the purpose of this analysis. IGF-I: insulin-like growth factor-I; IGFBP-3: insulin-like growth factor binding protein-3; IGF-I/IGFBP-3: their molar ratio, which provides a measure of free IGF-I; BMI: body mass index. * p<0.001

IGF-I, its binding proteins and ALS. IGF-I/IGFBP-3 ratio is reliable only in some conditions, such as acromegaly, GH deficiency etc. (Frystyk 2004). If there is a direct effect of IGF-I on the development of atherosclerosis, investigation of free IGF-I fraction is crucial. Unfortunately, there are minimal data on the association of free IGF-I with cardiovascular disease. Janssen *et al.* (1998) documented that low free IGF-I is associated with an increased risk of atherosclerosis in an elderly population. In another study (van der Beld *et al.* 2003), an inverse relation between carotid intima/media thickness and free (but not total) IGF-I was observed.

The inverse relationship between both total and LDL cholesterol and total IGF-I was originally observed in slightly hypercholesterolemic women (Prewitt *et al.* 1992). In a subsequent smaller study, total IGF-I was also inversely related to LDL-cholesterol (Ceda *et al.* 1998). In larger trials, this association was either neutral (Juul *et al.* 2002) or slightly positive (Laughlin *et al.* 2004). Positive correlation of total IGF-I with HDL-cholesterol and apolipoprotein A1 was reported (Ceda *et al.* 1998). Recent population studies found inverse relationships between total IGF-I and the extent of angiographically documented coronary atherosclerosis in men <70 years (Schuler-Luttmann *et al.* 2000). It was also shown that low total IGF-I significantly and independently increases the risk for subsequent ischemic heart disease (Juul *et al.* 2002), increases the cardiovascular mortality among elderly men and women (Laughlin *et al.* 2004), and is associated with the risk for the development of type-2 diabetes mellitus (Sandhu *et al.* 2002). The epidemiological studies did not specifically address the relationship between IGF-I and serum lipids. It was only observed that cardiovascular risk attributed to IGF-I was weaker after the adjustment for serum lipids (Juul and Gyllenborg 2003).

Our present and previous data (Malík *et al.* 2003) data suggest that the poorer prognosis of patients with reduced total IGF-I might partly result from the association with hyperlipidemia, which represents a significant risk factor itself. It is plausible to speculate that the mechanism, which underlies the reduction of total IGF-I, is different in IHC and MHL patients.

In patients with IHC, we observed similar levels of IGFBP-3 as in controls. Therefore, the reduction of IGF-

I/IGFBP-3 ratio in IHC patients cannot be explained by the change of IGF-binding capacity. Interestingly, in our preliminary study (Malík *et al.* 2003), IGFBP-3 was positively related to total cholesterol and IGFBP-3 levels were even significantly higher in hypercholesterolemic subjects. These findings may explain reduced free IGF-I, but not the total IGF-I decrease in IHC. There are only few studies which have examined the connection between cholesterol metabolism and GH/IGF-I axis. It was shown that both GH and IGF-I stimulate the density of hepatic LDL receptors (Rudling *et al.* 1992) and increase macrophage uptake and degradation of LDL (Hochberg *et al.* 1992). It was also reported that while native LDL increases the synthesis of IGF-I, oxidized LDL decreases production of both IGF-I and the IGF-I receptor (Scheidegger *et al.* 2000). In summary, the biological mechanism for decreased level of IGF in IHC remains elusive.

It should be emphasized that the MHL group differed from healthy controls not only by lipids, but also by other features of metabolic syndrome. Therefore, besides lipids, also other factors probably played a role in observed differences in IGF1 and IGFBP-3. In this group, we observed significantly lower IGFBP-3 than in controls. This difference was more pronounced than that of total IGF-I. Since IGFBP-3 carries more than 80 % of circulating IGF-I, this observation leads to a hypothesis that IGFBP-3 reduction is the primary, if not the only cause of total IGF-I lowering in MHL patients. Indeed, increased proteolysis of IGFBP-3 was observed in insulin resistance (Bang *et al.* 1994) that typically coexists with MHL. We can speculate that the proteolysis of IGFBP-3 might be a physiological mechanism of free IGF-I increase, because the main regulator of IGF-I, growth hormone, stimulates the liver production of not only IGF-I but also of IGFBP-3. On the other hand, presuming that only free IGF-I is a biologically active substance, the reduction of IGFBP-3 alone cannot explain the increase of free IGF-I. It may be that the increase of free IGF-I in MHL patients reflects an "IGF-I resistant" condition that parallels the hyperinsulinemia due to insulin resistance. Indeed, IGF-I and insulin have similar roles in glucose homeostasis, although they act through different receptors (Pratipanawatr *et al.* 2001, Sandhu *et al.* 2002). Of course, IGF-I/IGFBP-3 ratio is not the same as free IGF-I, but similar results as in our study were also reported by Ricart and Fernandez-Real (2001), who examined obese subjects with metabolic syndrome. The concept of IGF-I resistance was introduced by Pratipanawatr *et al.* (2001), who performed euglycemic IGF-I clamp. They found that in type 2 diabetic patients, the ability of IGF-I to augment total body glucose disposal and to suppress plasma free fatty acid concentration is impaired, indicating that both muscle and fat tissues are resistant to the action of IGF-I. In contrast, IGF-I keeps the ability to suppress hepatic glucose production also in diabetic subjects. Central IGF-I receptors are probably sensitive in insulin-resistant subjects as suggested by the following studies. Although the decrease of GH production was observed in obese subjects (Vahl *et al.* 1997), the responsiveness to external GH was high (Gleeson *et al.* 2005). Recombinant human IGF-I treatment of genetic forms of severe insulin-resistance resulted in increased insulin sensitivity (Moses *et al.* 1995). We could also speculate that the increase of free IGF-I in MHL is a natural mechanism to improve insulin sensitivity. Indeed, increased levels of portal insulin suppress hepatic production of another IGF-I binding protein, IGFBP-1 (not analyzed in this study) (Janssen *et al.* 1998). Furthermore, insulin *per se* increases liver synthesis of IGF-I *in vitro* (Schnetzler *et al.* 1991). We performed a *post hoc* selection of subjects with metabolic syndrome (according to ATPIII definition) from the MHL group and obtained similar results regarding to IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio as in the whole MHL group. Such findings should, however, be confirmed in another study, where metabolic syndrome (and not only MHL), should be the primary inclusion criterion.

Limitations

A general limitation of this and similar studies is that it is not possible to distinguish between effects of IGF-I and GH. These effects are counteracting in some aspects, such as in glucose regulation. Measurement of GH production is technically difficult due to large intra-day fluctuations of its level. Another limitation of these clinical studies is the inability to compare relative effects of ALS and of all binding proteins. However, the fact that IGFBP-3 binds over 80 % of serum IGF-I somewhat justifies this simplification.

Conclusions

This study demonstrates that hyperlipidemic patients have lower total IGF-I than normolipidemic subjects. The mechanism is presumably different in IHC and MHL. Because of a prominent reduction in IGFBP-3 in patients with MHL, they have reduced total IGF-I despite the actual elevation of IGF-I/IGFBP-3 ratio as a

possible surrogate of free IGF-I. This finding emphasizes the importance of clinical research focused on the role of free IGF-I in atherogenesis. An increase of free IGF-I in MHL patients may reflect an endogenous mechanism to improve insulin sensitivity and/or an "IGF-I resistant" condition that parallels the hyperinsulinemia of insulin resistance. The study suggests that differences in both IGF-I and its binding protein may represent an independent trait of patients with metabolic syndrome.

Conflict of Interest

There is no conflict of interest.

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