

ORIGINAL ARTICLE

Correlation of serum IGF-I and IGFBP-1 and -3 to cardiovascular risk indicators and early carotid atherosclerosis in healthy middle-aged men

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Summary

Objectives IGF-I, IGFBP-1 and IGFBP-3 are putative mediators in cardiovascular disease. The present study examined (i) the correlations of circulating IGF-I, IGFBP-1 and IGFBP-3 to established cardiovascular risk factors and signs of early atherosclerosis as reflected by ultrasound measurement of common carotid intima-media thickness (IMT), and (ii) whether serum concentrations of these analytes are modulated during alimentary lipaemia.

Design Cross-sectional clinical study.

Patients A biobank and clinical database based on 96 healthy Caucasian men, aged 50 years, with an apolipoprotein (apo) E3/E3 genotype, who had originally undergone investigations of postprandial lipoprotein metabolism was used for the study.

Measurements Total IGF-I, IGFBP-1 and IGFBP-3 were determined in serum by radioimmunoassay (RIA). Free IGF-I was measured by a commercial two-site immunoradiometric assay (IRMA).

Results In multivariate analyses, fasting serum free IGF-I correlated inversely with IMT and accounted for 5% of the variation in multiple R^2 . When fasting serum IGFBP-1 was entered in the models instead of IGF-I, IGFBP-1 correlated positively with IMT and accounted for 6% of the variation in IMT. IGFBP-3 and total IGF-I were unrelated to IMT. There were no associations between free IGF-I and cardiovascular risk factors, whereas IGFBP-1 behaved like a component of the insulin resistance syndrome. Serum free IGF-I increased and IGFBP-1 decreased postprandially.

Conclusion The data indicate that serum free IGF-I and IGFBP-1 are implicated in early atherosclerosis.

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Introduction

In the past few years IGF-I and two of its binding proteins, IGFBP-1 and IGFBP-3, have been identified as putative mediators in cardiovascular disease. IGF-I is a growth-promoting peptide that shares significant structural homology with insulin. In addition to endocrine effects, it has paracrine and autocrine activities. IGF-I production is regulated by growth hormone, insulin and nutrition.¹ Circulating IGF-I is mainly produced in the liver. However, IGF-I can be synthesized by many different cell types in almost all organs. Free IGF-I accounts for only 1% or less of total IGF-I. The remaining part is bound to one of six binding proteins (IGFBP-1 to -6), of which IGFBP-3 binds > 80% of circulating IGF-I.

IGF-I has many physiological functions. In the cardiovascular system it induces vascular smooth muscle cell (VSMC) proliferation and migration and suppresses VSMC apoptosis.² IGF-I also induces vasorelaxation and promotes endothelial cell migration in response to vascular injury.² In atherosclerotic lesions, IGF-I is considered to be important for monocyte chemotaxis, activation and cytokine release.² In addition, IGF-I interacts in glucose metabolism and increases insulin sensitivity.³

The IGF-I homeostasis is complex and the binding proteins form complexes with IGF-I to modulate its actions.⁴ IGFBP-3, which is regulated by GH and IGF-I, serves together with the acid labile subunit as the main reservoir for plasma IGF-I.⁵ IGFBP-1, however, is mainly regulated by insulin and is thought to be an acute regulator of IGF-I.⁶ Depending on the metabolic condition, IGFBP-1 either inhibits or potentiates IGF-I actions.^{4,6} IGFBP-1 has also been found to stimulate smooth muscle cell migration by an IGF-independent mechanism.⁷

In humans, low serum IGF-I has been associated with coronary artery disease (CAD) in both cross-sectional and prospective studies,⁸⁻¹⁰

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but the results are not consistent.^{11,12} The correlations of IGFBP-1 and -3 to cardiovascular disease have also been divergent. Both high and low circulating IGFBP-3 concentrations have been associated with CAD.^{9,12} In subjects with type 2 diabetes, low circulating IGFBP-1 concentration was associated with the presence of macrovascular disease,¹³ whereas no such association was found in an elderly healthy population.⁸ However, a low plasma or serum IGFBP-1 concentration has consistently been related to a less favourable cardiovascular risk profile in both healthy subjects and persons with type 2 diabetes.^{14–17} Low levels of IGF-I and IGFBP-1 have been reported to increase the risk of fatal CAD among elderly men and women, independently of established risk factors.¹⁸ In addition, an inverse association has been observed between levels of IGF-I and IGFBP-3 and risk of ischaemic stroke.¹⁹

The primary aim of the present study was to investigate the correlations of circulating IGF-I and two of its binding proteins (IGFBP-1 and -3) to established cardiovascular risk factors and signs of early atherosclerosis as determined by ultrasound measurement of the common carotid intima-media thickness (IMT). Particular emphasis was placed on the issue of whether IGF-I and IGFBP correlations with early atherosclerosis are independent of plasma lipoproteins. A second objective was to investigate whether serum concentrations of IGF-I (total and free), IGFBP-1 and IGFBP-3 are modulated during alimentary lipaemia and how they relate to postprandial lipaemia and subfractions of triglyceride-rich lipoproteins (TRLs). To reduce the confounding influence of age, sex and concomitant disease, the study group was restricted to population-based healthy men aged 50 years. Furthermore, only individuals with an apolipoprotein (apo) E3/E3 genotype (present in about 60% of the healthy Swedish population) were included because the apo E genotype influences the lipoprotein phenotype expression.

Materials and methods

Subjects

A biobank and clinical database comprising 96 Caucasian men, aged 50 years, living in the northern parts of the County of Stockholm was used in the study. These subjects had originally been recruited for studies of postprandial lipoprotein metabolism in relation to early signs of atherosclerosis.²⁰ They were selected randomly from a registry comprising all permanent residents. Inclusion criteria, in addition to male sex and age of 50 years, were North European or North American descent, the presence of an apo E3/E3 genotype and acquisition of technically satisfactory carotid ultrasound images. Exclusion criteria were chronic disease of any kind, a history of CAD or arterial thromboembolic disease, familial hypercholesterolaemia, body mass index (BMI) > 32 kg/m² and alcohol abuse. The study was approved by the Ethics Committee of Karolinska University Hospital, and all subjects gave their informed consent to participation.

Determination of IGF-I, IGFBP-1 and IGFBP-3

Total IGF-I was determined in serum by an in-house radioimmunoassay (RIA) after separation of IGFs from IGFbps by acid-ethanol extraction and cryoprecipitation. To minimize interference of

remaining IGFbps, des(1–3) IGF-I was used as a radioligand.²¹ The sensitivity of the RIA was 6 µg/l, and the intra- and interassay coefficients of variation (CVs) were 4% and 11%, respectively. Serum IGFBP-1 concentration was determined by RIA.²² The sensitivity of the RIA was 3 µg/l and the intra- and interassay CVs were 3% and 10%, respectively. IGFBP-3 in serum was determined by RIA using a commercially available kit (DSL 6700, Diagnostic System Laboratories, Webster, TX). Free IGF-I was measured by a commercial two-site immunoradiometric assay kit (DSL-9400 ACTIVE Free IGF-I IRMA kit, Diagnostic System Laboratories). The detection limit was 0.03 mg/l, the intra-assay CV 5.1% and the interassay CV 3.6%.

Glucometabolic and lipoprotein determinations

As described in greater detail elsewhere,²³ blood glucose was measured by a glucose oxidase method (Kodak Ektachem, Rochester, NY), and plasma insulin and intact proinsulin were determined by enzyme-linked immunosorbent assays (ELISAs) based on two monoclonal antibodies (DAKO Insulin and DAKO INTACT Proinsulin, Diagnostics Ltd, Cambridgeshire, UK). Plasma lipoproteins were quantified in the fasting state and after intake of a mixed meal. The methods used to determine plasma lipoprotein concentrations and the protocol of the oral fat tolerance test have been described previously.²⁰ In summary, TRLs were subfractionated by cumulative density gradient ultracentrifugation.²⁴ Consecutive runs calculated to float Svedberg flotation rate (Sf) 60–400 and Sf 20–60 particles were undertaken. The apo B-48 and apo B-100 concentrations (reflecting particle concentrations of intestinal- and liver-derived TRLs, respectively) were then determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in each fraction.²⁵ The test meal consisted of pasta, boiled drawn chicken breast meat, green peas and mayonnaise. The total energy content of the meal was 1000 kcal with 60 E% from fat, 13 E% from protein and 27 E% from carbohydrate. This corresponds to a fat load of approximately 65 g. The apo E genotype was determined using a polymerase chain reaction (PCR) technique.²⁶ The plasma low density lipoprotein (LDL) particle size distribution was determined by electrophoresis of LDL in 3–7.5% polyacrylamide gradient gels by which separation of LDL particles is achieved with high resolution.²⁷ C-reactive protein (CRP) concentration was determined by a high-sensitive particle-enhanced nephelometric immunoassay (Dade-Behring, Marburg, Germany).

Carotid artery ultrasound examinations

Carotid artery IMT was measured according to the European Lacidipine Study on Atherosclerosis ultrasound protocol.²⁸ The ultrasound instrument used was a Biosound 2000 II SA (Biosound, Inc., Indianapolis, IN) with an 8-MHz high-resolution annular array scanner. The scans were recorded on S-VHS videotapes and read at the Center for Medical Ultrasound, Division of Vascular Ultrasound Research, Wake Forest University, Winston-Salem, NC. In the present report, only the common carotid artery far wall IMT (mean of right and left artery registrations) was used as a measure of early atherosclerosis, and IMT henceforth refers to this segment of the carotid artery. The intrasonographer CVs were 3.8% and 5.1%, respectively, for the two sonographers. The intersonographer CV was 4.7%.

Statistical analyses

Distributions of serum or plasma determinations were expressed as median and interquartile range (IQR). The individual values of skewed variables were log-normalized before statistical tests. Responses of serum concentrations of IGF-I and IGFBPs to a mixed meal were analysed by an analysis of variance (ANOVA). Comparisons between measurements made at different time points during the test were made by the Scheffe *post hoc* test. Associations between clinical and metabolic variables and IMT were first assessed by calculation of univariate Pearson correlation coefficients. Multivariate models were subsequently generated by multiple linear regression analysis and multiple stepwise linear regression analysis to identify independent relationships between free IGF-I and IGFBP-1, on the one hand, and established clinical and metabolic risk indicators on the other, and variables independently correlating with IMT. A forward approach was used for the multivariate analysis, with significance levels set to less than 0.20 to enter and greater than 0.10 to leave the model. All statistical tests were two-sided and *P*-values of less than 0.05 were considered statistically significant.

Results

Basic characteristics and correlations with clinical risk factors

The basic clinical and metabolic characteristics of the 96 participants have been reported previously.^{20,23} The majority of the participants were nonobese [BMI, median (IQR) 25.2 (23.7–27.0) kg/m²], normotensive [systolic blood pressure 122 (115–130) mmHg; diastolic blood pressure 80 (75–85) mmHg] and had normal fasting blood glucose [4.7 (4.4–5.1) mmol/l] and insulin concentrations [32 (25–49) pmol/l] and normal fasting plasma lipid and CRP concentrations. The ultrasound examination revealed that, taken as a group, the 50-year-old men enrolled in the study had fairly normal IMTs [median (IQR) 0.85 (0.75–0.95) mm]. The total serum IGF-I concentration [median (IQR)] was 200 (163–254) ng/ml, whereas the serum concentration of free IGF-I was 0.39 (0.26–0.56) ng/ml. The corresponding concentrations of IGFBP-1 and IGFBP-3 were 20 (13–27) µg/l and 4.6 (3.6–6.3) mg/l, respectively. As shown in Table 1, there were no significant correlations between the serum concentrations of total IGF-I, free IGF-I and IGFBP-3, and waist to hip circumference ratio (WHR) or BMI. By contrast, IGFBP-1 was strongly inversely correlated with WHR and BMI. There was a strong inverse correlation between the serum concentrations of IGFBP-1 and free IGF-I ($r = -0.44$, $P < 0.01$), whereas the corresponding correlation between IGFBP-1 and total IGF-I was weaker ($r = -0.26$, $P < 0.05$). By contrast, serum IGFBP-3 was unrelated to total or free IGF-I.

Correlations between fasting serum concentrations of total and free IGF-I, IGFBP-1 and IGFBP-3 and glucose homeostasis, fasting plasma lipid and lipoprotein concentrations and CRP

There were strong inverse correlations between serum IGFBP-1 and plasma insulin and proinsulin concentrations (Table 1). Serum

Table 1. Correlations between fasting serum concentrations of total and free IGF-I, IGFBP-1 and IGFBP-3 and anthropometric parameters, glucometabolic variables, fasting plasma lipoproteins and CRP

	Total IGF-I	Free IGF-I	IGFBP-1	IGFBP-3
BMI	0.11	0.15	-0.44***	0.13
WHR	0.11	-0.01	-0.34***	-0.06
Glucose	-0.16	-0.02	-0.03	0.08
Insulin	-0.03	0.12	-0.47***	0.21*
Proinsulin	-0.02	0.12	-0.32**	0.20
Cholesterol				
VLDL	0.10	0.08	-0.21*	0.25*
LDL	0.04	0.06	0.01	-0.17
HDL	0.00	-0.04	0.28**	-0.29**
Triglycerides				
VLDL	0.07	0.08	-0.31**	0.21*
LDL peak particle size	-0.05	-0.11	0.23*	-0.25*
CRP	-0.16	0.07	-0.26*	-0.07

BMI, body mass index; WHR, waist to hip ratio; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein.

Values are univariate Pearson correlation coefficients.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

IGFBP-1 was inversely related to the plasma CRP concentration. In univariate analysis, IGFBP-1 was inversely correlated to the fasting plasma very low density lipoprotein (VLDL) triglyceride and VLDL cholesterol concentrations (Table 1). There was also a positive correlation between IGFBP-1 and the plasma high density lipoprotein (HDL) cholesterol concentration. IGFBP-3 showed an opposite pattern, with positive correlations to the plasma VLDL triglyceride and VLDL cholesterol concentrations and an inverse correlation to the plasma HDL cholesterol concentration. Both IGFBP-1 (positively) and IGFBP-3 (inversely) were correlated to LDL peak particle size, but not to the plasma LDL cholesterol concentration. By contrast, total and free IGF-I were unrelated to fasting plasma lipids. In multiple regression analysis, controlling for WHR and the plasma insulin concentration, all correlations between IGFBP-1 and fasting plasma lipids and LDL peak particle size and plasma CRP were lost (data not shown). Regarding subfractions of TRLs, IGFBP-1 was in univariate analysis inversely correlated to the fasting plasma concentrations of large VLDL particles (Sf 60–400 apo B-100) and large chylomicron remnants (Sf 60–400 apo B-48) (Table 2). No corresponding significant correlations were found for IGFBP-1 and small VLDL particles (Sf 20–60 apo B-100) or small chylomicron remnants (Sf 20–60 apo B-48). IGFBP-3, however, showed positive associations with the fasting plasma concentrations of all four TRL subfractions, but for large chylomicron remnants this was only of borderline statistical significance ($P = 0.06$). Total and free IGF-I showed no significant correlations with fasting plasma concentrations of TRLs. After controlling for WHR and the fasting plasma insulin concentration, all significant correlations between IGFBP-1 or IGFBP-3 and fasting plasma concentrations of large and small TRLs disappeared (data not shown).

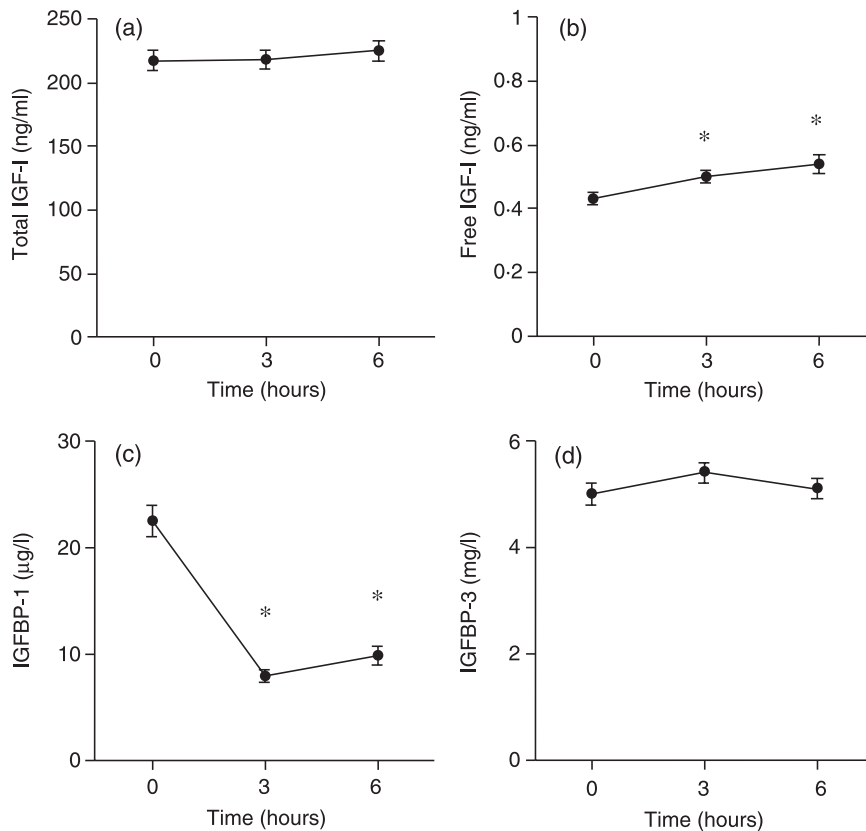


Fig. 1 Plots of the responses of serum concentrations of (a) total IGF-I, (b) free IGF-I, (c) IGFBP-1 and (d) IGFBP-3 to a mixed-meal type of oral fat tolerance test in 96 healthy 50-year-old men. Values are expressed as mean \pm SEM. * $P < 0.05$ compared with baseline.

Table 2. Correlations between serum concentrations of free IGF-I, IGFBP-1 and IGFBP-3 and alimentary lipaemia and fasting and postprandial plasma concentrations of triglyceride-rich lipoproteins

	Free IGF-I 0 h	IGFBP-1 0 h	IGFBP-3 0 h
tgAUC	0.05	-0.28**	0.20
Sf 60–400 apo B-100			
0 h	0.10	-0.28**	0.24*
3 h	0.10	-0.23*	0.19
6 h	0.08	-0.30**	0.21*
Sf 60–400 apo B-48			
0 h	0.02	-0.27**	0.19
3 h	-0.03	-0.12	0.15
6 h	0.02	-0.23*	0.28**
Sf 20–60 apo B-100			
0 h	-0.06	-0.04	0.20*
3 h	-0.03	0.01	0.13
6 h	0.01	-0.06	0.07
Sf 20–60 apo B-48			
0 h	-0.04	-0.14	0.20*
3 h	-0.11	-0.02	0.13
6 h	-0.05	-0.05	0.13

tgAUC, triglyceride area under the curve; Sf, Svedberg flotation rate; apo, apolipoprotein. Values are univariate Pearson correlation coefficients. 0 h designates baseline, and 3 h and 6 h the number of hours after intake of the test meal. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Postprandial responses of total and free IGF-I, IGFBP-1 and IGFBP-3 and their correlations to alimentary lipaemia and postprandial plasma concentrations of TRLs

The serum concentration of IGFBP-1 fell significantly after intake of the test meal, with the lowest value detected after 3 h (Fig. 1). By contrast, the postprandial serum concentration of free IGF-I increased (Fig. 1), whereas serum total IGF-I and IGFBP-3 did not change significantly from baseline. In the univariate analysis, the serum concentrations of IGFBP-1, at baseline and 3 h after intake of the test meal, were inversely related to the degree of postprandial triglyceridaemia (Table 2). This relationship was accounted for by correlations between IGFBP-1 and large VLDL particles (Sf 60–400 apo B-100) and large chylomicron remnants (Sf 60–400 apo B-48). IGFBP-3 showed a positive correlation to postprandial triglyceridaemia ($P = 0.05$ of 0 h), but the association with large VLDL particles and large chylomicron remnants tended to be weaker and less consistent compared with IGFBP-1 (Table 2). No correlations were found between IGFBP-1 and IGFBP-3 and postprandial plasma concentrations of small VLDL particles (Sf 20–60 apo B-100) or small chylomicron remnants (Sf 20–60 apo B-48). After adjustment for WHR and the fasting plasma insulin concentration, the associations between IGFBP-1 and postprandial triglyceridaemia and large VLDL particles and chylomicron remnants disappeared (data not shown), whereas significant correlations between serum IGFBP-1 at 3 h and small VLDL ($r = 0.23$, $P = 0.03$) and chylomicron remnants ($r = 0.30$, $P = 0.003$) at 3 h were apparent.

Table 3. Univariate and multivariate analyses of determinants of common carotid intima–media thickness including fasting serum free IGF-I

	Univariate analysis	Multiple regression		Multiple stepwise regression
	Pearson correlation coefficient	Regression coefficient	P-value	Increase in multiple R^2
WHR	0.11	0.004	0.99	NI
Systolic blood pressure	0.02	–0.000	0.88	NI
Cumulative tobacco consumption	0.16	0.000	0.64	NI
Alcohol	0.03	–0.000	0.95	NI
LDL cholesterol	0.23*	0.039	0.05	0.03
log proinsulin	0.26*	0.123	0.09	0.03
log TG at 2 h	0.31**	0.265	0.07	0.10
log Sf 60–400 apo B-100 at 3 h	0.21*	–0.119	0.23	NI
log free IGF-I 0 h	–0.19	–0.145	0.03	0.05
Multiple R^2				0.21

WHR, waist to hip circumference ratio; LDL, low density lipoprotein; TG, triglycerides; Sf, Svedberg flotation rate; apo, apolipoprotein; NI, not included. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4. Univariate and multivariate analyses of determinants of common carotid intima–media thickness including fasting serum IGFBP-1

	Univariate analysis	Multiple regression		Multiple stepwise regression
	Pearson correlation coefficient	Regression coefficient	P-value	Increase in multiple R^2
WHR	0.11	0.285	0.31	NI
Systolic blood pressure	0.02	–0.001	0.61	NI
Cumulative tobacco consumption	0.16	0.000	0.87	NI
Alcohol	0.03	0.000	0.98	NI
LDL cholesterol	0.23*	0.030	0.12	0.02
log proinsulin	0.26*	0.158	0.03	0.04
log TG at 2 h	0.31**	0.300	0.04	0.10
log Sf 60–400 apo B-100 at 3 h	0.21*	–0.123	0.21	NI
log IGFBP-1 0 h	0.15	0.166	0.01	0.06
Multiple R^2				0.22

WHR, waist to hip ratio; LDL, low density lipoprotein; TG, triglycerides; Sf, Svedberg flotation rate; apo, apolipoprotein; NI, not included. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Correlations of total and free IGF-I, IGFBP-1, IGFBP-3 and established risk factors to common carotid IMT

In the univariate analysis, there were no significant correlations between free IGF-I, total IGF-I, IGFBP-1 or IGFBP-3 and common carotid IMT. Otherwise, as reported previously,²⁰ LDL cholesterol, proinsulin, postprandial triglyceridaemia and postprandial plasma concentrations of large VLDL particles correlated positively and significantly with IMT (Tables 3 and 4). The relationship of free IGF-I to IMT was then explored by multivariate analyses (Table 3). In multiple regression analysis, LDL cholesterol and free IGF-I were significantly correlated with IMT. In a multiple stepwise regression analysis, plasma triglycerides at 2 h comprised the strongest determinant of IMT, accounting for 10% of its variation. Free IGF-I contributed to another 5% of the increase in multiple R^2 , whereas LDL cholesterol and proinsulin added 3% each. Thus, this model explained a total of 21% of the variation in IMT. Next IGFBP-1 was added and free IGF-I was removed (Table 4). In multiple regression analysis, proinsulin, plasma triglycerides at 2 h and IGFBP-1 were

significantly correlated with IMT. In multiple stepwise regression analysis, plasma triglycerides at 2 h remained the strongest determinant of IMT, accounting for 10% of its variation, whereas IGFBP-1 contributed to another 6%, proinsulin 4% and LDL cholesterol to 2% of the variation. Addition of plasma insulin to this model did not influence the association of IGFBP-1 with IMT, nor did insulin per se relate to IMT. In addition, inclusion of free IGF-I as a forced variable did not influence the relationship of IGFBP-1 to IMT (increase in multiple R^2 remained at 0.06). IGFBP-3 and total IGF-I did not contribute to the models when added in exchange for IGFBP-1 or free IGF-I. Postprandial plasma concentrations of IGF-I and the binding proteins were not explored in multivariate analysis as their univariate relationships with IMT were far from being statistically significant ($P > 0.20$).

Discussion

The objectives of the present study were to investigate the correlations of serum IGF-I (total and free) and two of its binding proteins

(IGFBP-1 and IGFBP-3) to signs of early atherosclerosis in a group of carefully characterized, middle-aged healthy men. We also analysed the postprandial responses of IGF-I, IGFBP-1 and IGFBP-3 and their correlations to postprandial lipaemia as we have demonstrated previously that postprandial triglyceridaemia is a fairly strong predictor of common carotid IMT.^{20,29}

The serum concentration of free IGF-I was found to be inversely and independently correlated with common carotid IMT after controlling for well-established risk factors for CAD and predictors of carotid IMT such as systolic blood pressure, tobacco consumption, plasma insulin/proinsulin and LDL cholesterol concentrations. However, no correlation was found between serum total IGF-I and IMT. A few recent studies have addressed the topic of IGF-I correlations to carotid IMT. In accordance with our results, van den Beld *et al.* found an inverse association between serum free IGF-I and carotid artery IMT in a group of elderly men,³⁰ irrespective of the presence of cardiovascular disease, whereas no such correlation was found for total IGF-I.³⁰ However, in a middle-aged to elderly population, hypertensive subjects who were genetically exposed to a low circulating total IGF-I concentration had increased carotid IMT compared with those with normal IGF-I levels,³¹ but in normotensives no such correlation was found. Unfortunately, free IGF-I was not measured. Finally, in a cross-sectional study of middle-aged to elderly subjects with and without cardiovascular disease, serum free IGF-I was found to be inversely associated with the presence of carotid plaques, but not with carotid IMT.¹⁶ Low serum IGF-I (free or total) has also been associated with cardiovascular disease in cross-sectional^{10,16} and prospective studies,^{9,18} and a genetically determined exposure to relatively low IGF-I levels is associated with increased risk of type 2 diabetes and myocardial infarction.³² Based on our observations and previous reports, we speculate that low serum levels of bioactive IGF-I may impair repair of early endothelial injury. It is possible, however, that later in the atherosclerotic process, high serum levels of bioactive IGF-I may stimulate VSMC proliferation, promoting progression of coronary atherosclerosis.¹¹

In our group of healthy middle-aged men, there were no significant correlations between serum total or free IGF-I and traditional cardiovascular risk factors, nor did we find any significant correlations with subfractions of TRLs in the fasting and postprandial state or with postprandial triglyceridaemia. This is in agreement with previous reports of no consistent associations between IGF-I and traditional cardiovascular risk factors.^{11,13,14,16,17} Thus, the independent association of serum free IGF-I with common carotid IMT and the absence of associations of free IGF-I with established cardiovascular risk factors suggest that IGF-I is directly implicated in atherogenesis. However, it should be emphasized in this context that common carotid IMT and clinical manifestations of cardiovascular disease cannot be equated.

By contrast, a low plasma or serum IGFBP-1 concentration has repeatedly been linked to components of the metabolic syndrome^{13–17} and IGFBP-1 is now considered as a robust marker of hepatic as well as whole-body insulin resistance. In our population, IGFBP-1 was also inversely correlated with BMI, WHR and fasting plasma concentrations of insulin, proinsulin, VLDL triglyceride and VLDL cholesterol, whereas positive correlations were found with LDL peak particle size and the HDL cholesterol concentration. In addition,

IGFBP-1 was inversely related to fasting and postprandial plasma concentrations of large VLDL particles and chylomicron remnants and to degree of postprandial triglyceridaemia. However, when controlling for insulin and WHR, all correlations with lipids and lipoproteins were lost, suggesting that a low serum IGFBP-1 concentration is a marker of insulin resistance and ensuing lipoprotein perturbations. Conversely, after adjustment for insulin and WHR, positive associations appeared between the serum IGFBP-1 concentration and the plasma concentrations of small VLDL and chylomicron remnants. This may reflect a greater generation of these lipoprotein species in insulin-sensitive individuals through more efficient lipolysis and/or direct synthesis.

IGFBP-1 was positively and independently associated with common carotid IMT in multivariate analysis. This is at first glance somewhat surprising because an inverse relationship would be expected when considering the known relationships between IGFBP-1 and traditional cardiovascular risk factors. However, the correlation between circulating IGFBP-1 and cardiovascular disease is obscure. In one recent study of persons with type 2 diabetes, serum IGFBP-1 was inversely correlated with carotid IMT, but the inverse correlation was not present in the subgroup without cardiovascular disease.³³ Others have failed to find any association between circulating IGFBP-1 and carotid IMT or cardiovascular disease.^{11,16,30} Two prospective studies have delivered contrary results regarding IGFBP-1 and mortality. In the first study, including elderly men, a high serum IGFBP-1 concentration was related to increased risk of cardiovascular mortality, irrespective of impaired glucose tolerance or diabetes, whereas no such correlation was found for total IGF-I or IGFBP-3.³⁴ In the other study, including elderly men and women, a low serum IGFBP-1 concentration was an independent risk factor for ischaemic heart disease mortality.¹⁸ Of note, it is generally believed that IGFBP-1 mainly inhibits the functions of IGF-I.² Accordingly, a high concentration of IGFBP-1 and a low serum concentration of free IGF-I would promote development of atherosclerosis, as was indicated in the present study. However, adjustment for free IGF-I in the multivariate analysis did not influence the positive relationship of IGFBP-1 to IMT, suggesting that there is a pro-atherogenic effect of IGFBP-1 that is independent of free IGF-I.

In people with manifest type 2 diabetes, insulin sensitivity is generally markedly reduced and the regulation of IGFBP-1 is likely to be perturbed. Furthermore, in the study by Leionen *et al.*, the plasma IGFBP-1 concentration was found to be dependent on the mode of diabetes treatment.³³ Importantly, these confounding factors were not present in the healthy population in our study.

Regarding the role of IGFBP-3, previous studies have presented divergent results. Both high⁹ and low¹² serum IGFBP-3 concentrations have been related to cardiovascular disease. In hypertensive patients, a high serum IGFBP-3 concentration was associated with the presence of carotid plaques but unrelated to mean carotid IMT.³⁵ We found no correlation between IGFBP-3 and common carotid IMT, in accordance with two published studies.^{30,33}

In the present study, postprandial changes in the serum concentrations of IGF-I (total and free), IGFBP-1 and IGFBP-3 were also investigated. As reported previously,⁴ IGFBP-1 fell significantly after intake of a test meal. Free IGF-I increased postprandially, whereas no significant changes were observed for total IGF-I or IGFBP-3. The

postprandial fall in serum IGFBP-1 concentration is most probably due to postprandial hyperinsulinaemia and the increase in free circulating IGF-I concentration secondary to acute regulation by IGFBP-1. As most of our time is spent in the postprandial state, it could be of value to determine free IGF-I after intake of a test meal in future epidemiological studies of the IGF-I axis in CAD.

Caution should be exercised when inferring causation from statistical associations obtained in a cross-sectional study, and it is also debatable to what extent serum levels of peptides contained in the GH/IGF-I axis accurately reflect their actions at the cellular level. These restrictions notwithstanding, we conclude, based on the results of the present study, that in the early stages of atherosclerosis elevation of circulating IGFBP-1 may reduce free IGF-I, with ensuing decreased capacity for vascular wall repair. This potential mechanism seems to be independent of traditional cardiovascular risk factors. In addition, IGFBP-1 may exert direct pro-atherogenic effects. IGFBP-3, however, does not seem to be directly implicated in early atherosclerosis. The exact mechanism behind the protective role of IGF-I in early stages of atherosclerosis remains to be revealed.

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