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Circulating insulin-like growth factor-I levels are correlated with the atherosclerotic profile in healthy subjects independently of age

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ABSTRACT. To investigate the relationships between the GH-IGF-I axis and the atherosclerotic profile, we designed this open, observational, prospective study. Peak GH after GHRH+arginine (ARG) test, serum IGF-I and IGF binding protein-3 (IGFBP-3), lipid profile, homeostasis model assessment (HOMA) index and intima-media thickness (IMT) at common carotid arteries were measured in 174 healthy individuals (92 women, 82 men, aged 18-80 yr). Exclusion criteria for this study were: 1) body mass index (BMI) ≥30 kg/m^2; 2) personal history of cardiovascular diseases; 3) previous or current treatments of diabetes or hypertension; 4) previous corticosteroids treatment for longer than 2 weeks or estrogens for longer than 3 months; 5) smoking of more than 15 cigarettes/day and alcohol abuse. Subjects were divided according to age in decade groups from <20 to >70 yr. BMI increased with age, as did systolic and diastolic blood pressures, although they remained in the normal range. The GH peak after GHRH+ARG test was significantly higher in the subjects aged <20 yr than in all the other groups (p<0.01), but was similar in the remaining groups. An inverse correlation was found between the IGF-I z-score and total/HDL-cholesterol ratio (p=0.02) and mean IMT (p=0.0009); IGFBP-3 z-score and mean IMT (p=0.043); IGF: IGFBP-3 molar ratio and total/HDL-cholesterol ratio (p<0.0001) and mean IMT (p<0.0001). Atherosclerotic plaques were found in 7 out of 12 subjects (53.8%) with a z-IGF-I score from ≤−2 to −1, in 4 out of 63 (6.3%) with a z-IGF-I score from −0.99 to 0.1 out of 66 (1.5%) with a z-IGF-I score from 0.1 to 1 and none of the 33 subjects with an IGF-I z-score >1 (p=0.006). At multi-step regression analysis, age was the best predictor of HDL-cholesterol levels and mean IMT, IGF-I level was the best predictor of total cholesterol and total/HDL-cholesterol ratio, the IGF-I/IGFBP-3 molar ratio was the best predictor of triglycerides levels. The z-scores of IGF-I and IGFBP-3 were the second best predictors of mean IMT after age. In conclusion, IGF-I and IGFBP-3 were negatively correlated with common cardiovascular risk factors, studied as total/HDL-cholesterol ratio, and/or early atherosclerosis, studied as IMT at common carotid arteries. The prevalence of atherosclerotic plaques, though not hemodinamically significant, was higher in the subjects having a z-score of IGF-I of ≤−2 to −1. Our results support a role of the IGF/IGFBP-3 axis in the pathogenesis of atherosclerosis.

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INTRODUCTION

IGF-I and its regulatory proteins, the IGF-binding proteins (IGFBPs), are growth promoters for arterial cells and mediators of cardiovascular disease (1). Deregulation of the IGFs, IGFBPs and IGFBP proteases axis causes detrimental effects on the vascular system by growth and migration of vascular smooth muscle cell, macrophage chemotaxis, excess LDL-cholesterol uptake, release of proinflammatory cytokines, migration and organization, forming capillary networks and synthesis of extracellular matrix in the atherosclerotic plaque (1). There is increasing evidence indicating that IGFs and their regulatory proteins, secreted by cells of the cardiovascular system, are growth promoters for arterial cells and mediators of cardiovascular diseases (2, 3).

Key-words: IGF-I, atherosclerosis, common carotids, intima-media thickness, lipid profile.

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The existence of a relationship between the IGF-I system and atherosclerosis was also supported by investigations in adult hypopituitary patients with GH deficiency (GHD) (4-10) or acromegaly (11-19). GHD patients were shown to have increased intima-media thickness (IMT) at major arteries with increased prevalence of atherosclerosis which is, at least partially, reversed by GH replacement treatment (6, 7). We have also demonstrated similar negative atherosclerotic profile in patients with hypopituitarism and GHD, showing that only the patients with IGF-I levels below the normal range had values of IMT at common carotids higher that their matched controls (10). On the contrary, patients with acromegaly do not have increased prevalence of atherosclerotic plaques and only mild increase of common carotid IMT (11, 12), which is reduced by suppressing GH and IGF-I levels (13, 14). Both the patients with acromegaly and those with GHD have endothelial dysfunction (15-17).

In recent years, three independent cohort studies performed in Denmark and the USA have associated IGF-I levels in the lower part of the normal range with an increased risk of ischemic heart disease (IHD) in patients not bearing pituitary diseases (18-20). Juul et al. (18) showed that subjects in the low IGF-I quartile had significantly higher risk of IHD with a relative risk (RR) of 1.94 compared with the high IGF-I quartile group. Individuals in the high IGFBP-3 quartile group had an adjusted RR of 2.16 of having IHD (18). Identification of a high-risk population with low IGF-I and high IGFBP-3 levels resulted in markedly higher risk of IHD (RR 4.07) compared with the index group (18). Vasan et al. (19) in the community-based, prospective cohort study of Framingham showed that there was a 27% decreased risk for heart failure for every 1 SD increment in log IGF-I. Laughlin et al. (20) in the community-based, prospective cohort study of Rancho-Bernardo showed that IGF-I and IGFBP-1 were independently and jointly related to risk of IHD mortality: RR of IHD mortality was 38% higher for every 40 µg/l [1 SD] decrease in IGF-I and 3.1 times greater for those in the lowest quintile of IGFBP-1 compared with those with higher IGFBP-1 levels (20). IGF-I and IGFBP-1 (alone or in combination) were not related to risk of all cause or non-IHD mortality (20). The important conclusion of all these studies is that serum IGF-I levels are inversely related to the risk for congestive and IHD.

In order to determine the relationships between the GH-IGF-I axis and the atherosclerotic profile, we measured the peak GH after GHRH+arginine (ARG) test, serum IGF-I and IGFBP-3, lipid profile and IMT at common carotid arteries in a large series of healthy individuals aged 18-80 yr and free of any disease of the cardiovascular system.

SUBJECTS, MATERIALS AND METHODS

Subjects

One-hundred and seventy-four healthy subjects (92 women and 82 men, aged 18-80 yr), among the clerks, medical and paramedical personnel of the Department of Molecular and Clinical Endocrinology and Oncology of the "Federico II" University of Naples, and their relatives agreed to participate in this study. Exclusion criteria for this study were: 1) body mass index (BMI) ≥30 kg/m^2; 2) personal history of cardiovascular diseases; 3) previous or current treatments with drugs known to interfere with glucose or lipid metabolism or to influence blood pressure; 4) previous treatment with corticosteroids for longer than 2 weeks; 5) previous or current treatment with estrogens for longer than 12 weeks; 6) smoking of more than 15 cigarettes/day and alcohol abuse (more than 3 glasses of wine/day). Smoking was stratified into the following: 1) non-smokers; 2) ex-smokers; 3) mild smokers (up to 15 cigarettes/day). According to Juul et al. (18) physical activity was stratified into the following: 1) mostly sedentary; 2) mild exercise (walking, bicycling or other, at least 4 h/week); and 3) demanding exercise or heavy activity during leisure hours for at least 4 h/week. The characteristics of the subjects at study entry according to their age are shown in Table 1. All subjects gave their informed consent to participate in this study that was designed in accordance with the Helsinki II Declaration on human experimentation.

Study design

It was an open, observational, prospective study.

Study protocol

In all subjects the GH peak after GHRH+ARG test were measured, according to Aimaretti et al. (21), serum IGF-I and IGFBP-3, total cholesterol, HDL-cholesterol and triglycerides levels after an overnight fasting. The total/HDL-cholesterol ratio was also calculated as an index of cardiovascular risk (22). Within 7-15 days from endocrine and metabolic evaluation, all subjects underwent common carotid arteries ultrasonography. Heart rate, systolic and diastolic blood pressure (SBP and DBP, respectively) were measured before starting the procedure. Blood pressure was measured at the right arm, with the subjects in a relaxed sitting position. The average of six measurements (three taken by each of two examiners) with a mercury sphygmomanometer was used for analysis. The fourth Korotkoff phase was considered as DBP. Hypertension was diagnosed in the presence of DBP above 90 mmHg (23): none of the subjects had stable hypertension, which was an exclusion criterion. Hypertriglyceridemia was diagnosed when triglyceride levels were >150 mg/dl (1.7 mmol/l) (24), while hypercholesterolemia was diagnosed when total cholesterol levels were >200 mg/dl (5.2 mmol/l) (25). The estimate of insulin resistance was calculated by the homeostasis model assessment (HOMA) score, by applying the Matthews' et al. formula (26):

[fasting serum insulin (µU/ml) x fasting plasma glucose (mmol/l)/22.5].

The conversion factors (mg/dl to mmol/l) for lipids and glucose were as follows: cholesterol 0.02586, triglycerides 0.01129, and glucose 0.05551.

Carotid ultrasonography

Common carotid arteries ultrasound imaging was carried out with a Vingmed Sound CMF 725 equipment (Horten, Norway)

Table 1 - *Subject profile according to age.*

		<20 yr	21-30 yr	31-40 yr	41-50 yr	51-60 yr	61-70 yr	71-80 yr	*p*
No. (female/male)		20 (11/9)	35 (19/16)	21 (11/10)	33 (18/15)	25 (13/12)	21 (10/11)	19 (10/9)	
Median age (yr)		19	24	36	46	57	66	76	
Smoking [no. (%)]	1	15 (75)	23 (66)	12 (57)	14 (42)	9 (36)	4 (19)	6 (32)	0.001
	2	0 (0)	0 (0)	1 (5)	14 (42)	6 (24)	14 (67)	12 (63)	<0.0001
	3	4 (25)	12 (34)	8 (38)	5 (16)	10 (40)	3 (14)	1 (5)	0.033
Physical activity [no. (%)]	1	9 (45)	18 (51)	14 (67)	29 (88)	20 (80)	20 (95)	18 (95)	<0.0001
	2	11 (55)	15 (43)	4 (19)	4 (12)	4 (16)	1 (5)	1 (5)	<0.0001
	3	0 (0)	2 (6)	3 (14)	0 (0)	1 (4)	0 (0)	0 (0)	<0.0001
BMI (kg/m^2)		22.1±2.6	23.0±2.2	24.2±3.0	24.1±2.8	24.1±2.9	25.0±2.4	26.4±2.2	<0.0001
GH peak after GHRH+ARG test (μg/l)		73.4±28.5	49.9±19.9	46.1±15.7	52.8±17.2	51.7±17.6	43.6±14.9	42.1±13.2	<0.0001
Z-score IGF-I		–0.20±0.57	0.05±0.62	–0.16±0.69	0.71±0.82	0.74±0.90	0.31±0.68	–0.23±0.84	<0.01
Z-score IGFBP-3		–0.46±0.8	–0.12±0.77	0.28±0.95	0.48±0.89	0.76±0.84	0.42±0.69	0.21±1.15	<0.01
Total cholesterol levels (mg/dl)		166.6±12.8	171.8±17.6	170.0±17.9	180.5±16.7	181.8±21.1	188.2±19.2	198.7±17.7	<0.0001
HDL-cholesterol levels (mg/dl)		67.6±5.8	61.3±5.4	59.2±7.6	60.7±6.6	60.5±7.7	58.6±5.2	54.3±3.6	<0.0001
Total/HDL-cholesterol ratio		2.47±0.26	2.83±0.57	2.86±0.44	3.02±0.53	3.06±0.64	3.24±0.54	3.68±0.49	<0.0001
Triglycerides levels (mg/dl)		76.1±8.1	92.7±21.6	88.2±22.9	97.6±17.4	99.2±21.1	103.1±22.4	123.5±23.6	<0.0001
HOMA index		1.27±0.60	1.31±0.58	1.81±1.09	1.28±0.51	1.66±0.80	1.24±0.48	1.90±0.45	<0.01
SBP (mmHg)		115.3±9.2	121.3±7.6	119.0±13.0	123.2±7.5	119±13.8	125.2±15.8	147.4±11.9	<0.0001
DBP (mmHg)		74.5±6.8	77.6±5.3	77.9±6.6	79.7±4.7	75.2±7.8	80.7±4.8	85.0±4.4	<0.0001
Heart rate (bpm)		77.8±5.1	78.1±5.2	78.6±7.0	78.2±5.5	78.3±7.5	80.0±4.9	78.4±5.8	0.93
IMT at right common carotid (mm)		0.55±0.07	0.59±0.07	0.63±0.10	0.69±0.11	0.71±0.13	0.78±0.07	0.90±0.09	<0.0001
IMT at left common carotid (mm)		0.55±0.07	0.59±0.07	0.64±0.11	0.68±0.08	0.71±0.13	0.82±0.14	0.91±0.10	<0.0001
Right systolic peak velocity (cm/sec)		57.4±4.0	63.8±8.7	64.5±9.9	72.8±9.3	75.4±5.2	81.2±5.3	84.7±4.4	<0.0001
Left systolic peak velocity (cm/sec)		58.2±4.8	64.1±8.3	64.2±10.3	71.3±8.8	76.3±5.5	80.1±5.9	85.4±5.4	<0.0001
Right diastolic peak velocity (cm/sec)		12.1±2.2	14.8±3.1	16.3±3.0	17.4±3.4	18.3±2.6	20.9±2.8	23.5±2.8	<0.0001
Left diastolic peak velocity (cm/sec)		12.6±3.1	14.9±3.0	16.1±3.4	17.1±3.3	19.0±2.9	20.3±3.1	23.5±3.2	<0.0001

p values refer to the results of analysis of variance (ANOVA) among groups. Smoking was stratified into the following: 1) non-smokers, 2) ex-smokers, 3) mild smokers (up to 15 cigarettes/day). Physical activity was stratified into the following: 1) mostly sedentary, 2) mild exercising (walking, bicycling or otherwise at least 4 h/week) and 3) demanding exercising or doing heavy activity during leisure hours for at least 4 h/ week.
SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HOMA: homeostasis model assessment; BMI: Body mass index; IMT: intima-media thickness; ARG: arginine.

by means of a 7.5 MHz annular phased array transducer. Details on the technique were reported elsewhere (10, 11, 13, 14). We measured the IMT at the right and left common carotid artery wall and calculated the average mean IMT value for individual subjects as well. The IMT variability of measurement for our instrument was 0.03 mm. Our intra-observer variability for repeated measurements of carotid artery diameter is 0.01±0.02 mm. Flow indices of both carotids were investigated by measuring blood systolic (SPV) and diastolic peak velocities (DPV). Presence, location and size of plaques were also evaluated at the level of common carotid arteries. A type IV plaque featured by thickening of vascular wall and increased density of all US-detectable layers without any hemodynamic alteration was defined as a well-defined plaque (27). All measurements were made by one investigator (S.S.) to minimize the intra-observer variability.

Assays

All hormone measurements were performed using the same reagents at the Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University of Naples. Serum GH levels were measured by immunoradiometric assay (IRMA) using commercially available kits (HGH-CTK-IRMA Sorin, Saluggia, Italy). The sensitivity of the assay was 0.2 μg/l. The intra- and inter-assay coefficients of variation (CVs) were 4.5 and 7.9%, respectively. Serum IGF-I

was measured by IRMA after ethanol extraction using Diagnostic System Laboratories Inc. (Webster, Texas, USA). The normal range in ≤20, 21-30, 31-40, 41-50, 51-60, 61-70 and >70-yr-old men was 180-625, 118-475, 102-400, 100-306, 95-270, 88-250, 78-200 µg/l, respectively, whereas in women it was 151-530, 118-450, 100-390, 96-288, 90-250, 82-200, 68-188 µg/l, respectively. The sensitivity of the assay was 0.8 µg/l. The intra-assay CVs were 3.4, 3.0 and 1.5% for low, medium and high points of the standard curve, respectively. The inter-assay CVs were 8.2, 1.5 and 3.7% for low, medium and high points of the standard curve. Plasma IGFBP-3 was measured by radioimmunoassay (RIA) after ethanol extraction using Diagnostic System Laboratories Inc. (Webster, Texas, USA). The normal range in ≤20, 21-30, 31-40, 41-50, 51-60, 61-70 and over 70-yr-old subjects was 2.1-7.6, 1.9-6.6, 1.7-6.3, 1.6-5.4 1.6-4.3, 1.6-4.0 and 1.5-3.8 mg/l, respectively. The sensitivity of the assay was 0.5 µg/l. The intra-assay CVs were 3.9, 3.2 and 1.8% for low, medium and high points of the standard curve, respectively. The inter-assay CVs were 0.6, 0.5 and 1.6% for low, medium and high points of the standard curve. The IGF-I/IGFBP-3 molar ratio was estimated in order to get a better understanding of the relative concentration changes of IGF-I and IGFBP-3 levels. The values for the molecular mass of IGF-I and IGFBP-3 used for the calculation were: 7649 and 28,500 Da, respectively (28). Fasting total, LDL- and HDL-cholesterol, triglycerides and fibrinogen levels were measured by standard procedures.

Statistical analysis

Results were expressed as median or mean±SD unless otherwise specified. The statistical analysis was performed by SPSS Inc. (Cary, NC) package using parametric or non-parametric tests, according to the distribution of individual variables. The comparison across different age groups was made by the analysis of variance (ANOVA). The significance was set at 5%. Categorical variables were compared using Pearson's chi-square test. Correlation coefficients were calculated by measuring Pearson's coefficient. The stepwise multiple linear regression was performed to evaluate the relative importance of age, BMI, peak GH after GHRH+ARG, IGF-I levels, z-score of IGF-I, IGFBP-3 levels, z-score of IGFBP-3 and the IGF-I/IGFBP-3 molar ratio on total cholesterol levels, HDL-cholesterol levels, the total/HDL-cholesterol ratio, triglycerides levels, HOMA index, right and left IMT, mean IMT, right and left SPV and DPV. In this analysis, we entered only those variables that had a p value of less than 0.01 in the univariate analysis.

RESULTS

Age and BMI vs *GH response to GHRH+ARG and the IGF-I axis*

Table 1 shows patients' distribution according to age stratified by decades. Smoking was not frequent in our population: 84 subjects were non-smokers (48.3%), 43 were smokers (24.7%) of a median of 10 cigarettes/day and 47 were ex-smokers (27%). Ex-smokers were significantly older than non-smokers and smokers (61.2±11.8 *vs* 37.3±17 and 40.4±16.8 yr, p<0.0001) so that comparison with the other two groups was biased by age. When comparing smokers with non-smokers, we did not find any difference in the peak GH after GHRH+ARG (49.4±19.0 *vs* 54.8±21.9 µg/l, p=0.17), z-IGF-I score (0.24±0.78 *vs* 0.27±0.8, p=0.84), total/HDL-cholesterol ratio (2.93±0.61 *vs* 2.85±0.51, p=0.43), HOMA index (1.52±0.66 *vs* 1.41±0.73, p=0.41) or mean IMT (0.66±0.14 *vs* 0.64±0.12 mm, p=0.43). The vast majority of our subjects had a sedentary life-style and only the young subjects (<30 yr) regularly exercised (Table 1). As expected, BMI increased with age, as did SBP and DBP, although remaining in the normal range. Only the group aged >70 yr had an average BMI above the threshold of normal weight (≤25 kg/m^2). The GH peak after GHRH+ARG test was significantly higher in the subjects aged <20 yr than in all the other groups (p<0.01) and was similar in the remaining groups. Both IGF-I and IGFBP-3 z-scores varied in different groups: the subjects aged 41-50 and 51-60 yr had average IGF-I z-scores that were significantly higher than all the other groups (p<0.01), and the subjects aged 41-50, 51-60 and 61-70 yr had average IGFBP-3 z-scores that were significantly higher than all the other groups (p<0.01).

Lipid profile vs *GH response to GHRH+ARG and the IGF-I axis*

Total-cholesterol, the total/HDL-cholesterol ratio and triglycerides levels significantly increased across different age groups while HDL-cholesterol levels fell significantly (Table 1). A significant inverse correlation was found between the IGF-I z-score and total cholesterol (r=–0.19, p=0.01), total/HDL-cholesterol ratio (r=–0.18, p=0.02) and triglycerides levels (r=–0.19, p=0.01). No correlation was found between IGF-I z-score and HDL-cholesterol levels as well as between IGFBP-3 z-score and lipid profile. The IGF/IGFBP-3 molar ratio was significantly correlated with total cholesterol (r=–0.34, p<0.0001), HDL-cholesterol (r=0.37, p<0.0001), total/HDL-cholesterol ratio (r=–0.41, p<0.0001, Fig. 1A) and triglycerides levels (r=–0.39, p<0.0001).

IMT and atherosclerotic plaques vs *GH response to GHRH+ARG and the IGF-I axis*

As expected, at right and left common carotid arteries IMT, SPV and DPV significantly increased with age (Table 1). Type IV plaques without significant hemodynamic alterations were found in 13 subjects (7.5%): 7 women (7.6%) and 6 men (7.3%), of whom 3 aged 51-60 yr, 4 aged 61-70 yr and 6 aged more than 70 yr. Both IGF-I (Fig. 2A) and IGFBP-3 z-scores (Fig. 2B) were significantly correlated with IMT, as well as the IGF-I/IGFBP-3 ratio (Fig. 1B). None of our subjects had an IGF-I score of <−2 associated with an IGFBP-3 score of >2. Based on the stronger correlation of IGF-I z-score on IMT (Fig. 2A), we grouped the patients according to an IGF-I z-score in four groups: 1) z-score from ≤−2 to −1 (no.=12);

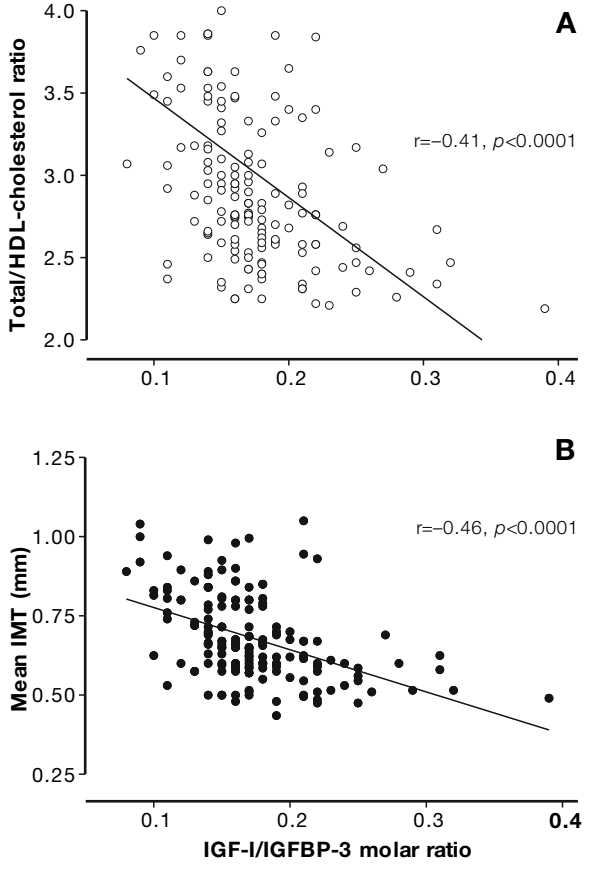

Fig. 1 - *Results of the correlation analysis between the IGF/IGFBP-3 molar ratio and the total/HDL-cholesterol ratio (A) and the average value of intima-media thickness (IMT) measured at the right and left common carotid arteries (CCA) - mean IMT (B) - in the 174 subjects.*

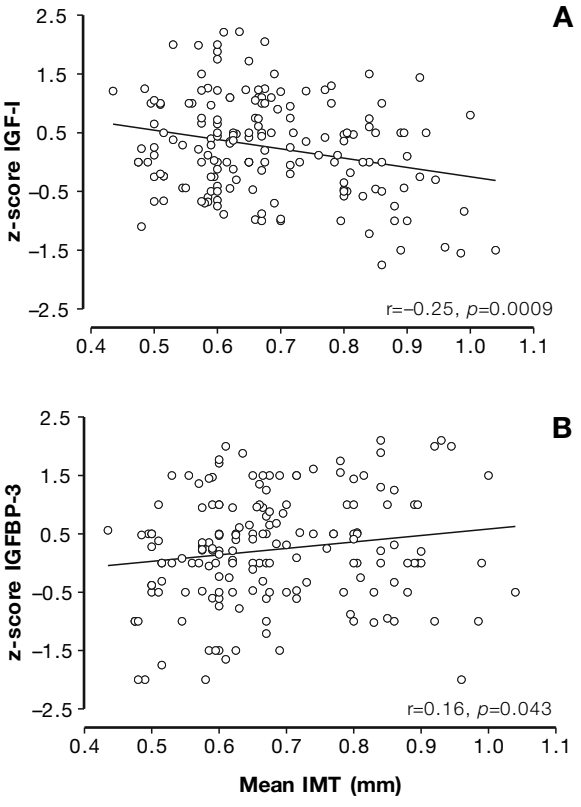

Fig. 2 - *Results of the correlation analysis between the average value of intima-media thickness (IMT) measured at the right and left common carotid arteries (mean IMT) and the z-score of IGF-I (A) and the z-score of IGFBP-3 (B) in the 174 subjects.*

2) from −0.99 to 0 (no.=63); 3) from 0.1 to 1 (no.=66) and 4) from 1.1 to ≥2 (no.=33). As shown in Table 2, the 12 subjects with the lowest IGF-I z-score were slightly older than the other groups, had no change in BMI or heart rate while they did have higher blood pressure values and a more compromised lipid and vascular profile than all the other groups. Individual values of IMT at right and left common carotid arteries according to the IGF-I z-score are shown in Figure 3. Atherosclerotic plaques were found in 7 subjects of group 1 (53.8%), 4 of group 2 (6.3%), 1 of group 3 (1.5%), and none of group 4 (p=0.006).

Multi-step regression analysis (Table 3)

Age was the best predictor of HDL-cholesterol levels and mean IMT, IGF-I level was the best predictor of total cholesterol and total/HDL-cholesterol ratio, the IGF-I/IGFBP-3 molar ratio was the best predictor of triglyceride levels. The GH peak after GHRH+ARG was the second best predictor of total cholesterol, HDL-cholesterol, total/HDL-cholesterol ratio, and triglyceride levels. The z-scores of IGF-I and IGFBP-3 were the second best predictors, after age, of mean IMT at common carotid arteries.

DISCUSSION

This open, observational, prospective study shows that the GH/IGF-I system is closely linked to the atherosclerotic profile in non hypopituitary subjects. The different subset of the system, *i.e.* the GH peak after GHRH+ARG, IGF-I z-score, IGFBP-3 z-score and the IGF-I/IGFBP-3 molar ratio, were all related to the lipid profile and the IMT at common carotid arteries. Interestingly, a lower IGF-I z-score was significantly associated with an increased prevalence of atherosclerotic plaques in subjects without apparent increased cardiovascular risk, *i.e.* normal weight, normal blood pressure, normal lipid profile and non-heavy smokers before entering the study.

Table 2 - *Subjects profile according to the z-score of IGF-I.*

	From ≤–2 to –1	From –0.99 to 0	From 0.01 to 1	From 1.01 to ≥2	*p*
No. (W/M)	12 (4/8)	63 (33/30)	66 (32/34)	33 (23/10)	
Median age [yrs (range)]	64 (20-80)*	34 (18-80)	42.5 (19-79)	49 (22-74)	0.001
BMI (kg/m^2)	24.7±2.9	23.9±2.9	24.4±2.5	23.3±3.1	0.13
GH peak after GHRH+ARG test (μg/l)	36.7±18.1	52.9±24.7	52.9±18.4	50.8±12.7	0.04
Total cholesterol levels (mg/dl)	207.9±25.3**	177.6±18.0	177.8±18.5	173.4±16.0	0.0006
HDL-cholesterol levels (mg/dl)	54.6±4.6***	61.4±6.9	59.9±6.6	62.0±7.3	0.018
Total/HDL-cholesterol ratio	3.75±0.78**	2.96±0.59	3.00±0.56	2.83±0.43	0.0005
Triglycerides levels (mg/dl)	123.2±24.3***	96.5±23.0	95.2±23.2	90.9±16.9	0.003
HOMA index	1.60±0.50	1.51±0.70	1.51±0.29	1.28±0.51	0.13
SBP (mmHg)	143.8±10.5**	121.3±7.6	119.0±13.0	123.2±7.5	<0.0001
DBP (mmHg)	84.6±3.3**	77.6±5.3	77.9±6.6	79.7±4.7	0.002
Heart rate (bpm)	77.2±2.1	78.1±5.2	78.6±7.0	78.2±5.5	0.39
IMT at right common carotid (mm)	0.86±0.18**	0.68±0.15	0.67±0.13	0.64±0.10	0.002
IMT at left common carotid (mm)	0.88±0.19**	0.69±0.15	0.68±0.14	0.64±0.09	0.001
SPV at right common carotid (cm/sec)	83.0±8.5**	68.1±11.9	70.9±11.0	70.5±9.0	0.0008
SPV at left common carotid (cm/sec)	84.5±9.1**	68.7±11.9	70.5±10.5	69.6±9.2	0.0004
DPV at right common carotid (cm/sec)	21.7±4.8***	16.9±4.5	17.3±4.1	16.6±3.7	0.012
DPV at left common carotid (cm/sec)	23.0±4.8**	16.7±4.7	17.2±4.0	16.8±3.3	0.002

*: $p<0.01$ *vs* group 2; **: $p<0.01$ *vs* all other groups; ***: $p<0.05$ *vs* all other groups. BMI: body mass index; ARG: arginine; HOMA: homeostasis model assessment; SBP: systolic blood pressure; DBP: diastolic blood pressure; IMT: intima-media thickness; SPV: systolic peak velocity; DPV: diastolic peak velocity.

Three independent observational prospective studies have associated low levels of IGF-I with an increased risk of IHD and even death due to heart failure in middle-aged to elderly subjects not bearing nether pituitary diseases nor cardiac diseases at the time of blood sampling (18-20). More recently, Fisher et al. (29) reported that the presence of coronary heart disease (CHD) was found to be significantly positively associated with circulating levels of total IGF-I, IGFBP-5, acid-labile subunit (ALS) and IGFBP-3 in a case-control, cross-sectional study involving non-diabetic male patients. Interestingly, the associations between CHD and the IGF axis were independent of traditional risk factors, insulin and sex hormones (29). In this latter study, however, CHD patients had significantly higher serum concentrations of IGF-I, IGFBP-3, IGFBP-5 and ALS than age- and BMI-matched controls selected from the random working population. Nonetheless, Fisher et al. (29) also concluded that there is a high likelihood of a pathogenetic role of the GH/IGF axis in coronary atherosclerosis. Increased levels of IGF-I were also reported by Ruotolo et al. (30) in young male survivors of myocardial infarction. In analogy with the previously reported epidemiological studies (18-20), other studies found that IGF-I levels were lower in patients with angiographically documented CHD than in matched controls (31) or in elderly patients developing cardiovascular disease (32).

In our cohort of subjects undergoing endocrine, metabolic and ultrasonographic screening in clinically healthy conditions and excluding common factors, such as diabetes, hypertension, smoking and alcohol consumption able *per se* to increase the prevalence of atherosclerosis, we found a significant negative correlation between circulating levels of IGF-I, IGFBP-3 and their molar ratio with lipid profile and common carotid IMT. In particular, in our series we carefully excluded all the subjects with diabetes or glucose intolerance as the association of IGFs with cardiovascular diseases might also be a surrogate for the actions of insulin (33). Insulin-resistant individuals are frequently overweight or obese and have various disturbances of the endocrine system, also possibly including low levels of GH and

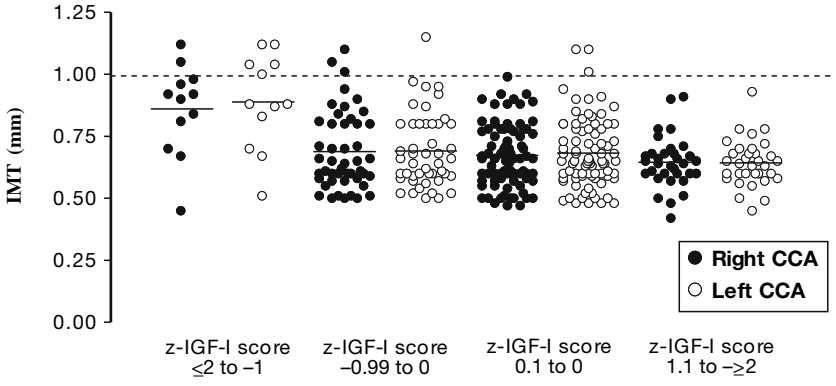

Fig. 3 - *Individual values of intima-media thickness measured at the right and left common carotid arteries (CCA) in the 174 subjects grouped according to their z-score of IGF-I. (For the statistical significance aming groups see Table 2). IMT: intima-media thickness.*

IGF-I (34). In our series, all subjects had a normal weight with the exception of slightly higher BMI levels in those aged above 70 yr: none of them, however, was obese. According to our strict inclusion criteria, there was no difference in insulin resistance according to the HOMA method among different groups in accordance with z-IGF-I scores. In our series, we found significantly higher GH peak after GHRH+ARG test in subjects aged <20 yr compared to all the other groups. This is partially in disagreement with the results reported by Ghigo et al. (35) who did not report age-difference in the response to this test in the adult population. It should be noted that in the study by Ghigo et al. (35) the number of subjects aged between 18 and 20 yr was not quoted and, however, young adults were few. Interestingly, the mean IMT value was predicted in our series firstly by age and secondly by the z-scores of IGF-I and IGFBP-3, again supporting a pathogenetic role of this axis in the development of atherosclerosis. These results were in line with those reported by Juul et al. (18) in another cohort of healthy subjects. As a further support to the role of IGF-I and IGFBP-3 in the atherosclerotic process, none of the subjects having a z-IGF-I score >1 had evidence of atherosclerotic plaques, while 53.8% of those with a z-IGF-I score from ≤−2 to −1 had atherosclerotic plaques.

Table 3 - *Stepwise regression analysis.*

Best predictor(s)		β Coefficient	t	*p*
Total cholesterol levels	IGF-I	–0.49	–7.65	<0.0001
	GH peak	–0.22	–3.33	<0.0001
HDL-cholesterol levels	Age	–0.25	–3.38	0.001
	GH peak	0.19	2.60	0.010
	BMI	–0.20	–2.50	0.010
Total/HDL-cholesterol ratio	IGF-I levels	–0.391	–5.85	<0.0001
	GH peak	–0.174	–2.59	0.011
	BMI	0.139	2.01	0.046
	IGF-I/ IGFBP-3 MR	0.135	1.99	0.048
Triglyceride levels	IGF-I/ IGFBP-3 MR	–0.410	–5.99	<0.0001
	GH peak	–0.139	–2.04	0.043
	IGFBP-3 levels	–0.313	–4.81	0.0001
Mean IMT	Age	0.654	11.96	<0.0001
	z-sds IGF-I	–0.500	–6.09	<0.0001
	z-sds IGFBP-3	0.275	2.79	0.006

BMI: body mass index; IGFBP: IGF binding protein; MR: molar ratio. *IMT: mean value of intima-media thickness measured at the right and left common carotid arteries.

The relationships between the IGF axis and atherosclerosis are complex and probably change according to healthy or disease status. On the one hand, IGF-I is potentially proatherogenic, mainly by stimulating vascular smooth muscle cell proliferation and extracellular matrix synthesis in the atherosclerotic plaque (1-3), while on the other hand it improves endothelial function by stimulating nitric oxide synthesis (36), so reducing IMT and plaque formation, also demonstrated in hypopituitary GHD patients during GH replacement (6, 7). In an attempt to oversimplify the effects of the IGF axis on vascular physiology, the balance between the pro-atherogenic and anti-atherogenic effects of IGF-I could explain the contrasting results reporting the association between low IGF-I levels and later development of CHD/IHD in subjects not having pituitary diseases (18-20) and the cardiovascular/cerebrovascular mortality in hypopituitary patients (37-40) as well as the association of high IGF-I levels in patients after CHD (29, 30) or increased IMT at major arteries in acromegaly (11-14). Additionally, as vascular smooth muscle cells in the intimal region of advanced atherosclerotic plaques show low expression of IGF-I and IGF-I receptor, IGF-I was argued to act as a survival factor for vascular smooth muscle cells which prevent plaque instability and rupture (41).

In conclusion, in a large series of healthy subjects, aged 18-80 yr, we found a negative correlation of IGF-I, IGFBP-3 and their molar ratio with the common cardiovascular risk factors studied as total cholesterol, HDL-cholesterol, total/HDL-cholesterol ratio and triglycerides, and early atherosclerosis, studied as IMT at common carotid arteries. The prevalence of atherosclerotic plaques, though not hemodinamically significant, was found to be significantly higher in the subjects having a low z-IGF-I score of ≤−2 to −1. Atherosclerotic plaques were absent in the subjects having a z-IGF-I score of >1. Our results support a role of the IGF/IGFBP-3 axis in the pathogenesis of atherosclerosis.

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