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The Effect of *GHR*/exon-3 Polymorphism and Serum GH, IGF-1 and IGFBP-3 Levels in Diabetes and Coronary Heart Disease

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Abstract. *Aim: The present study investigated the effects of growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3) and GH-receptor (GHR)/exon-3 polymorphism on diabetes mellitus (DM) and coronary heart disease (CHD) patients. Patients and Methods: Ninety patients with CHD, 90 patients with DM and 96 controls were included in this study. The GH, IGF-1 and IGFBP-3 serum levels were measured with enzyme-linked immunosorbent assay. GHR/exon-3 variants were determined by multiplex-polymerase chain reaction. Results: The frequency of all alleles and genotypes in all study groups were distributed according to the Hardy-Weinberg equilibrium. In addition, any association between GHR/exon-3 variants and the presence of risk factors were detected. The blood levels of GH, IGF-1 and IGFBP-3 were not distributed according to GHR/exon-3 variants. However, in the DM group, higher levels of IGF-1 and lower levels of GH and IGFBP-3, and in CHD group lower levels of IGF-1, GH and IGFBP-3 were observed. The order of GH levels were DM<CHD< Controls; IGF-1 levels were CHD<Controls<DM and IGFBP-3 levels were CHD<DM<Controls. Conclusion: No direct effect of GHR/exon-3 polymorphism was observed in DM or CHD patients. However GH, IGF-1, IGFBP-3 and insulin were thought to act together to establish body homeostasis in patients with DM and CHD.*

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Diabetes mellitus (DM) (1, 2) and coronary heart disease (CHD) (3-5) both have higher prevalences worldwide and are multifactorial diseases in which many genes and environmental factors contribute to their aetiopathogenesis.

The growth hormone (GH) regulates lipid, carbonhydrate, protein and mineral metabolisms and, due to its importance in metabolic control, studies are accelerated in determining the possible effects of GH on the aetiology of diabetes and atherosclerosis (6-8). As a diabetogenic molecule, GH would be suspected to generate insulin resistance in tissues like adipose, muscle and liver, which respond to both GH and insulin (9, 10, 12). Moreover, liver, adipose tissue and muscle have shown to induce insulin resistance by GH (11, 12). On the other hand, the hypothesis that GH deficiency increases the risk of CHD is supported by a series of risk factors, which were also the distinct markers of the accelerated atheromatosis, such as adverse lipid profiles, increased blood pressure, abnormal body composition, increased body weight, increased coagulability and increased inflammation markers. However, these effects could be normalized with GH replacement therapy (13, 15).

GH is synthesized and secreted by the somatotropic cells of the anterior pituitary and shows its anabolic effects by inducing the proliferation and growth *via* activating its peripheric cell surface receptor, GHR. Insulin-like growth factor-1 (IGF-1), which is synthesized by liver and some peripheric local cells, mediates the effects of GH. However, 90% of the circulating IGF-1 was held by insulin-like growth factor binding protein-3 (IGFBP-3) and this performs an increase in the half-life of IGF-1. On the other hand, IGFBP-3 is not only a carrier molecule, it also regulates the

biological activity of IGF-1 and acts as a growth inhibitor, independently from IGF-1 (16-20).

As GH signals *via* GHR and induces a variety of cellular events, the polymorphisms on the *GHR* gene affects these cellular events (16-21). In humans, the *GHR* gene is composed of 9 exons and has two common variants depending on the expression of exon 3 (full length, GHR-fl and exon 3-deleted, GHR-d3) (17).

In the present study, due to the controversial results of prior studies, the blood levels of GH, IGF-1, IGFBP-3, as well as the effects and distribution of *GHR*/exon-3 polymorphism in a Turkish population of diabetic or coronary heart disease patients are investigated in order to understand the complex interactions of these factors that may be beneficial for prevention and therapy strategies for diabetes mellitus and coronary heart diseases.

Patients and Methods

Patient selection and clinical investigation. Ninety patients diagnosed with CHD at the Marmara University, Department of Cardiovascular Surgery, 90 patients diagnosed as DM at the Istanbul University, Department of Internal Medicine, Division of Diabetes and 96 healthy volunteers as controls were included in the study.

Severe coronary heart disease patients were documented by angiography. The inclusion criteria were 50% or more stenosis of at least one major coronary vessel with atherosclerotic plaque or a vascular event, which is defined as myocardial infarction or performed percutaneous transluminal coronary angioplasty or coronary artery by-pass grafting. Type 2 diabetes was diagnosed according to American Diabetes Association criteria (21). The control group consisted of healthy subjects without any symptoms of both DM and CHD. However, the presence of early atherosclerotic coronary artery lesions could not be excluded in the control group due to the lack of coronary angiography.

The study protocol was approved by both the Ethical Committee of the Istanbul Faculty of Medicine and the Research Fund of Istanbul University. All participants in the study signed informed consent forms in accordance with ethics guidelines regarding the study.

GH, IGF-1 and IGFBP-3 measurement. The GH, IGF-1 and IGFBP-3 serum levels were measured with enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen, Camarillo, CA 93012 USA for GH; Mediagnost, D-72770 Reutlingen, Germany for IGF-1 and IGFBP-3), according to the manufacturer's instructions.

Multiplex polymerase chain reaction (PCR)-based detection of GHR/exon-3 genotypes. Blood specimen were collected in tubes containing EDTA and genomic DNA samples were extracted from leukocyte nuclei by an Invitrogen iPrep™ Purification Instrument with genomic DNA isolation kit (iPrep™ PureLink™ gDNA Blood Kit; Life Technologies, Carlsbad, CA 92008 USA).

The DNA samples were analyzed for the *GHR*/exon-3 polymorphism by multiplex PCR with locus-specific primers as previously reported (22, 23). After the amplification of *GHR*/exon-3 locus, both the presence and size of these DNAs were confirmed by 2% agarose gel electrophoresis.

Statistical methods. Statistical analysis was performed with the SPSS software package (revision 21; SPSS Inc., Chicago, IL, USA). The Hardy-Weinberg equilibrium was tested for all polymorphisms. Clinical laboratory data are expressed as mean±standard deviation (SD). Mean values were compared between patients and controls by unpaired the Student's *t*-test. Differences in the distribution of genotypes and alleles between cases and controls were tested using the Chi-square-statistic and Fisher's-exact tests. Allele frequencies were estimated by gene counting methods. Values of $p<0.05$ were considered statistically significant.

Results

The baseline characteristics of the study population and the distribution of GH, IGF-1 and IGFBP-3 in the study groups are shown in Table I. As expected, both CHD and DM patients showed significantly higher prevalences of traditional risk factors with respect to healthy subjects. In the DM group, higher levels of IGF-1 ($p>0.05$) and lower levels of GH ($p<0.001$) and IGFBP-3 ($p<0.001$), and in the CHD group lower levels of IGF-1 ($p<0.001$), GH ($p>0.05$) and IGFBP-3 ($p<0.001$) were observed. The order of GH levels were DM<CHD<Controls; IGF-1 levels were CHD<Controls<DM and IGFBP-3 levels were CHD<DM<Controls.

The *GHR*/exon-3 genotype and allele distribution is shown in Table II. The frequency of all alleles and genotypes in all study groups were distributed according to the Hardy-Weinberg equilibrium ($p>0.05$). In addition, any association between *GHR*/exon-3 variants and the presence of risk factors as hypertension, obesity, critical levels of plasma lipid profiles, cigarette or alcohol consumption ($p>0.005$) was detected. However, the prevalence of the body mass index (BMI) ≥27kg/m^2 was higher in CHD patients possessing the *GHR*-d3/d3 genotype than those carrying the *GHR*-fl allele (*GHR*-fl/fl+GHR-fl/d3 genotype) ($p=0.087$).

In Table III, the distributions of clinicopathological parameters according to *GHR*/exon-3 variants are presented. In diabetics, the homozygous *GHF*-fl allele carriers have higher systolic blood pressure (SBP) than *GHR*-d3 allele carriers ($p=0.025$). In addition, lower plasma IGF-1 and glucose, and higher GH and IGFBP-3 levels were detected in GHR-fl allele (*GHR*-fl/fl+*GHR*-fl/d3) carriers than those having the *GHR*-d3/d3 genotype ($p>0.05$) (Tables III and IV). When the ANOVA statistical test was performed for analyzing these parameters among the *GHR*/exon-3 genotypes, it was seen that the *GHR*-d3/d3 genotype carriers have higher plasma levels of glucose than the other genotypes, especially than the *GHR*-fl/d3 genotype ($p=0.065$). In CHD patients, there was no significant difference among clinicopathological parameters ($p>0.005$). The serum GH, IGF-1 and IGFBP-3 levels according to *GHR*/exon-3 variants are presented in Table IV. The highest serum levels of GH and IGFBP-3 were seen in the

Table I. *Baseline characteristics of the study population.*

		Study groups			*p*-Values		
		Control (n=96)	DM (n=90)	CHD (n=90)	1 p	2 p	3p
Gender (n,%)							
Male		51 (%53.1)	27 (%30.0)	68 (%75.6)	**0.001**	**0.001**	**<0.001**
Female		45 (%46.9)	63 (%70.0)	22 (%24.4)			
Age		64.05±9.89	54.98±9.34	63.71±8.82	**<0.001**	0.805	**<0.001**
BMI (kg/m^2)		25.82±3.64	32.51±6.39	27.30±3.16	**<0.001**	**0.004**	**<0.001**
≥27 kg/m^2		24 (%25.0)	75 (%83.3)	46 (%51.1)	**<0.001**	**<0.001**	**<0.001**
<27 kg/m^2		72 (%75.0)	15 (%16.7)	44 (%48.9)			
TC (mg/dl)		177.99±26.58	195.04±31.74	198.11±34.64	**<0.001**	**<0.001**	0.537
TG (mg/dl)		121.70±54.24	163.48±82.74	155.34±46.64	**<0.001**	**<0.001**	0.418
HDL-C (mg/dl)		49.65±12.97	46.54±11.44	44.28±30.80	0.086	0.120	0.516
LDL-C (mg/dl)		100.98±23.47	115.54±28.59	124.91±35.67	**<0.001**	**<0.001**	**0.054**
VLDL-C (mg/dl)		27.47±16.67	30.84±16.30	38.59±28.51	0.166	**0.001**	**0.026**
HbA1c		5.91±0.44	7.83±1.59	8.45±6.44	**<0.001**	**<0.001**	0.377
Glucose (mg/dl)		94.71±9.36	181.73±60.67	135.70±54.27	**<0.001**	**<0.001**	**<0.001**
SBP (mmHg)		124.39±11.97	136.23±20.52	143.39±19.91	**<0.001**	**<0.001**	**0.019**
DBP (mmHg)		75.14±7.80	84.03±11.38	82.59±10.75	**<0.001**	**<0.001**	0.383
GH (ng/ml)		1.77±1.68	0.95±1.36	1.69±1.68	**<0.001**	0.766	0.001
IGF1 (ng/ml)		492.82±222.92	500.14±176.76	277.82±178.93	0.805	**<0.001**	**<0.001**
IGFBP3 (ng/ml)		8499.50±2406.56	3108.51±965.96	2204.48±1343.28	**<0.001**	**<0.001**	**<0.001**
Cigarette (n, %)	(+)	15 (%15.6)	27 (%30.0)	41 (%45.6)	**0.019**	**<0.001**	**0.031**
	(–)	81 (%84.4)	63 (%70.0)	49 (%54.4)			
Alcohol (n, %)	(+)	-	11 (%12.2)	12 (%13.3)	**<0.001**	**<0.001**	0.823
	(–)	96 (%100)	79 (%87.8)	78 (%86.7)			

CHD, Coronary heart disease; DM, diabetes mellitus; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high- density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; VLDL-C, very-low-density lipoprotein-cholesterol; HbA1c, hemoglobinA1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP-3, IGF binding protein-3. The results are shown as mean±SD. n, number of individuals; $p<0.05$ and bold values indicate statistical significance. p1, Control *vs*. DM; p2, Control *vs*. CHD; p3, DM *vs*. CHD.

control group, whereas the highest IGF-1 level was found in diabetics than the other study groups. In addition, the serum levels of GH, IGF-1 and IGFBP-3 were also analyzed according to the genotypes of *GHR*/exon-3 polymorphism. In the control group, the order of serum GH levels was GHR-fl/d3>GHR-d3/d3>GHR-fl/fl and the IGF-1 and IGFBP-3 levels were *GHR*-d3/d3>*GHR*-fl/fl>*GHR*-fl/d3. When the statistical significance was analyzed in this group, it was seen that only the heterozygous genotype carriers (GHR-fl/d3) have significantly highest serum GH levels than those carrying homozygous wild (*GHR*-fl/fl) or mutant genotype (*GHR*-d3/d3) (p=0.044 and 0.076, respectively). In diabetic patients, although no statistical association was found, the order of GH levels were *GHR*-fl/fl>*GHR*-d3/d3>*GHR*-fl/d3; IGF-1 levels were *GHR*-d3/d3>*GHR*-fl/fl>*GHR*-fl/d3 and IGFBP-3 levels were *GHR*-fl/d3>*GHR*-fl/fl>*GHR*-d3/d3 ($p>0.05$). Finally, in the CHD group, the order of GH and IGF-1 levels were *GHR*-fl/d3>*GHR*-d3/d3>*GHR*-fl/fl and IGFBP-3 levels were *GHR*-d3/d3>*GHR*-fl/d3>GHR-fl/fl ($p>0.05$).

Table II. *The distribution of GHR/exon-3 genotypes and allele frequencies in the study groups.*

	Control (n=96)	DM (n=90)	CHD (n=90)
Genotypes			
GHR-fl/fl	42 (%43.8)	49 (%54.4)	40 (%44.4)
GHR-d3/d3	35 (%36.5)	34 (%37.8)	41 (%45.6)
GHR-fl/d3	19 (%19.8)	7 (%7.8) *	9 (%10.0)
Alleles			
GHR-fl allel	103 (%53.7)	105 (% 58.3)	89 (%49.4)
GHR-d3 allel	89 (%46.3)	75 (%41.7)	91(%50.6)

CHD, Coronary heart disease; DM, diabetes mellitus; n, number of individuals; *$p<0.05$.

Discussion

Studies focused on CHD, one of the macrovascular complications of diabetes, revealed that several risk factors are involved in its aetiology but none of them are efficient to explain

Table III. *Comparison of background characteristics among GHR/exon-3 genotypes in the study groups.*

	Control			DM			CHD		
	fl/fl genotype (n=42)	d3 allele (d3/d3+fl/d3) (n=54)	*p*-Value	fl/fl genotype (n=49)	d3 allele (d3/d3+fl/d3) (n=41)	*p*-Value	fl/fl genotype (n=42)	d3 allele (d3/d3+fl/d3) (n=54)	*p*-Value
Age (year)	63.57±10.59	64.43±9.39	0.677	55.59±7.85	54.27±10.92	0.506	63.35±9.49	64.00±8.35	0.731
BMI (kg/m^2)	26.43±3.46	25.36±3.75	0.157	32.59±7.04	32.42±5.60	0.899	27.57±3.32	27.10±3.05	0.490
TC (mg/dl)	179.97±28.01	176.44±25.57	0.521	198.16±28.06	191.31±35.65	0.311	203.11±39.09	194.10±30.45	0.222
TG (mg/dl)	125.71±60.57	118.57±49.12	0.525	166.69±92.18	159.63±70.77	0.689	148.00±36.14	161.22±53.22	0.183
HDL-C (mg/dl)	50.02±12.76	49.35±13.24	0.803	46.41±10.56	46.71±12.54	0.902	41.12±19.01	46.82±37.69	0.386
LDL-C (mg/dl)	102.45±23.95	99.83±23.06	0.590	118.90±25.47	111.54±31.79	0.226	129.94±38.85	120.89±32.75	0.234
VLDL-C (mg/dl)	27.93±18.62	27.13±15.15	0.817	30.37±11.38	31.41±20.86	0.763	39.94±26.69	37.51±27.78	0.690
HgA1c	5.87±0.45	5.95±0.45	0.354	7.71±1.60	7.97±1.63	0.439	7.95±4.58	8.84±7.63	0.517
Glucose (mg/dl)	95.81±9.15	93.85±9.51	0.312	177.02±54.23	187.37±67.82	0.424	137.30±44.74	134.44±61.26	0.806
SBP (mmHg)	125.83±13.23	123.26±10.88	0.298	140.63±21.92	130.98±17.54	**0.025**	140.28±15.84	145.88±22.50	0.170
DBP (mmHg)	74.40±7.96	75.70±7.70	0.421	85.88±12.02	81.83±10.29	0.093	82.28±11.24	82.84±10.44	0.806

CHD, Coronary heart disease; DM, diabetes mellitus; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; VLDL-C, very-low-density lipoprotein-cholesterol; HbA1c, hemoglobinA1c; SBP, systolic blood pressure; DBP, diastolic blood pressure. The results are shown as mean±SD. n, number of individuals; bold values indicates statistical significance ($p<0.05$).

Table IV. *Serum levels of GH, IGF-1 and IGFBP-3 according to GHR/exon-3 variants.*

	Group	Genotypes			Alleles	
		GHR-fl/fl	*GHR*-d3/d3	*GHR*-fl/d3	*GHR*-fl	*GHR*-d3
GH (ng/ml)	Control	1.55±1.17	1.64±1.88	2.49±2.12	1.84±1.57	1.94±1.99
	DM	1.08±1.71	0.91±0.72	0.23±0.45	0.97±1.63	0.80±0.73
	CHD	1.51±1.32	1.79±1.71	2.08 ±2.82	1.61±1.67	1.84±1.92
IGF-1 (ng/ml)	Control	493.20±245.75	504.70±209.88	470.03±201.65	485.99±231.48	492.51±205.78
	DM	502.44±188.25	516.42±152.50	404.89±199.79	490.25±190.63	497.38±164.25
	CHD	260.76±156.49	284.17±198.87	324.68±186.90	272.50±162.31	291.46±195.53
IGFBP-3 (ng/ml)	Control	8490.00±2420.38	8710.00±24.43.37	8132.00±2391.03	8378.62±2397.11	8506.00±2418.48
	DM	3119.40±970.57	3050.18±1010.13	3315.62±788.86	3143.93±945.67	3095.50±972.30
	CHD	1954.71±1019.57	2426.61±1545.89	2302.66±1575.29	2018.62±1129.93	2404.30±1535.68

CHD, Coronary heart disease; DM, diabetes mellitus; GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP-3, IGF binding protein-3. The results are shown as mean±SD.

the pathogenesis of disease alone. It is a complex disease with high-cost due to cooperativity of genetic and environmental factors. Recent studies emphasize that genetic factors, including polymorphisms in genes encoding proteins, such as GH receptors, IGFs and IGFBPs located on the GH axis, may have roles in the development of DM and CHD beside the traditional risk factors (9-13). GH released from the anterior lobe of the hypophysis, mediating post-natal growth and development in mammals, has stimulatory effects on anabolism in the muscular and bony tissues and inhibitory effects on the lypolysis in the adipose tissue as well. Furthermore, it promotes glucose uptake into the muscle cells. Although it is well-documented that the first target of GH is the induction of growth and development by stimulation of IGF-1 synthesis in the liver, it was also shown to have a direct local effect on the development of target tissues by promoting synthesis of IGF-1 (24).

The biological functions of GH are achieved by expression of target genes (*IGF-1, C-FOS, C-JUN, CYP2/3, JUN-B*) resulting from activation of STAT-1, STAT-3 and STAT-5 through phosphorylation of relevant proteins at the inner side of the cell membrane upon binding to special GHRs. IGF-1 is also released into the circulation by IGFBPs, which is synthesized under the control of GH and, especially, in an IGFBP3-dependent manner. In this way, keeping constant IGF-1 levels in the circulation, as well as prolonged half-life and circulatory depots of IGF-1, will be established (25).

There exist several studies reporting that GH has both diabetic and anti-diabetic effects in the carbohydrate metabolism (26, 27). High doses of GH have been shown to induce resistance against the effects of insulin on glucose metabolism and contribute to lipid oxidation as well (26). According to studies on dogs and cats, the increment of blood glucose levels by application of GH is considered as a diabetic effect (28). In contrast, studies on adipose tissues from mice and hens revealed that GH has an anti-diabetic feature because it facilitated glucose uptake into these tissues (29). Due to complex and opposing effects of GH in diabetes and, thus, in the pathogenesis of CHD, the levels of GH, IGF-1 and IGFBP-3 in the GH-axis -together with the polymorphisms in the *GHR* gene- are all candidate genes for atherosclerosis and related diseases.

Our results, obtained for GH, IGF-1 and IGFBP-3 levels by *GHR*/exon-3 deletion polymorphism in DM and CHD, partly correlate with the results of previous studies. The discordance between these studies and our study may be due to size differences between groups, differences in ethnical traits and differences of environmental risk factors, such as smoking and nutritional habits in the population. Furthermore, contrary results in the literature may originate from the differing effects of insulin sensitivity created by GH, a molecule that is diabetogenic and anti-diabetogenic as well.

Verhelst and Abs, Beshyah and Johnston and Stewart and Sheppard reported that obesity is an important health problem in GH-deficient individuals (13, 30, 31). It is well known that high circulatory insulin levels (hyperinsulinaemia) leads to obesity and, in the long run, diabetes by creating insulin resistance and beta-cell disfunctioning; this will be in turn followed by CHD, which is one of the serious diabetic macrovascular complications. Studies on such diseases revealed that high IGF-1 levels (as its expression depends on GH) trigger obesity and that regulation of insulin resistance by GH drew attention to a regulation between insulin and GH release. Accordingly, insulin has inhibitory actions on GH release from the hypophysis and produces peripheral effects by increasing IGF-1 levels through inhibition of IGFBP production in the liver. High free IGF-1 levels were reported to suppress GH release by its negative feed-back mechanism and consequently cause development of insulin resistance resulting in high levels of glucose, amino acids and fatty acids in the plasma (14). Thomas and Monson and Monson *et al*. reported high prevalence of diabetic individuals suffering from hypophysial hypofunction due to GH insufficiency (32, 33). Besides, several reports confirm development of insulin resistance in untreated GH deficiencies. Thus, it was accepted that cardiovascular events, in addition to obesity, generate more risk in individuals with GH deficiency due to hypophysial hypoactivity (14, 34-37).

Many studies showed that the effects of GH replacement therapy against insulin resistance appeared to be biphasic as the initial increase in risky states due to high glucose and insulin levels was followed by normalisation of expected insulin levels by improvement of sensitivity against insulin during long-term treatments (10, 34, 36, 37). Conversely, there are studies showing both worsening or improvement of glucose tolerance (38, 39) by long-term GH treatments. In placebo-controlled studies, it was reported that short-term GH-replacement improves systolic (30) and diastolic (27) functions and low dose treatments appear to act positively on cardiac performance (40). Too high doses, however, reported to generate cardiac risk by ventricular deterioration, especially by contribution to left ventricular hypertrophy (27, 34). Khan *et al*. reported that GH may cause non-specific effects due to its antigenic and pro-lactogenic features; high doses of GH may also contribute to acromegalic side-effects, insulin resistance and genesis of cardiac diseases (41).

Although all functions of GH and its blood levels under physiological conditions depend on its interaction with GHR, the gene for *GHR* shows genetic polymorphism and may have many variants. Pantel *et al*. showed exon-3 deleted isoform coded by the exon-3 deleted *GHR* allele and reported that possession of either *GHR*-fl or *GHR*-d3 allele is sufficient for normal growth (22, 23). However Santos *et al*. reported that GHR-d3 was biologically more active than the *GHR*-fl isoform (18). Audi *et al*. compared short-height and normal-height individuals and found that the frequency of biologically less active *GHR*-fl/fl genotype in short-height group is higher than in the normal-height group (42).

In our study, while GH and IGFBP-3 levels were found to be lower and IGF-1 higher in diabetics, in CHD patients, these parameters were found to be at low levels with respect to controls. These results are thought to be within compensatory limits and could be governed by an inter-relationship between insulin and GH/IGF-1/IGFBP-3 cycles. Indeed, for the maintenance of normal physiologic balance, glucose homeostasis is mediated by GH and insulin together with opposing effects on the regulation of blood glucose levels (14, 26, 27). In normal healthy individuals, GH release is depressed by hyperglycaemia, physical stress, high blood levels of free fatty acid, obesity and senescence, whereas it is increased by fasting and loss of body weight in order to establish nitrogen balance and to protect body proteins. Both GH and IGF-1 are kept under strict control by negative feed-backs between these agents (14, 24, 25).

In diabetes, the insulin level reducing blood glucose is low. Therefore, low levels of insulin, which has a direct inhibitory effect on the hypophysis, leads to increased GH and IGFBP levels; this, in turn, promotes synthesis and/or release of IGF-1 in the liver and in tissues; which tends to

reduce blood glycose to normal levels by creating an insulin-like effect. On the other hand, high IGF-1 levels may cause hypoglycaemia by profoundly reducing blood glycose and inhibit GH release, which stimulate its own synthesis and release by a negative feedback (24, 25) mechanism. Thus, the low level of GH and high level of IGF-1 in diabetic patients is, in fact, a compensatory state that efficiently explains the contradictory reports in the literature concerning the increase or decrease of GH and IGF-1 levels in DM. Besides, the IGFBP-3 protein level, which also contributes to the regulation of hypoglycaemia by binding to high IGF-1 levels, was found to be lower in diabetics. This situation was also thought to be compensatory because, under normal conditions, IGF-1 was found to be by 20% in its free form and 80% complexed with IGFBP-3, which serves as a depot for IGF-1. Therefore, high levels of IGF-1 in DM can bind more IGFBP-3, thus causing a decrease in IGFBP-3 levels.

Our results indicate, that slight GH and low IGF-1 and IGFBP-3 levels in CHD, are thought to be due to normal or high insulin levels. In CHD, cardiovascular damage, including endothelial dysfunction in tissues and organ systems, are far in excess. Therefore, it is vital to keep insulin levels at normal or high levels in order to protect tissues from the harmful effect of glucose. Although this harmful effect may be compensated by high levels of GH with stimulation of protein synthesis, this may also paradoxically increase blood glucose levels. In addition, the extent of damage may also be worsened by inducing proliferation in endothelial and smooth muscle cells through production of surplus amount of elastin, proteoglycane and collagen in the vascular medial layer. Therefore, it is ideal to let IGF free from its complex form by inhibition of IGFBPs rather than to stimulate new IGF synthesis. Both the synthesis and release of GH and IGFBP-3 are inhibited by high plasma insulin levels. As free IGF-1 level increases as a result of reduction of IGFBP-3 levels this elevated IGF-1 undergo tissue repair by increasing protein synthesis at the site of damage. Thus, IGF-1 synthesis may be kept at a low level as it is not synthesised due to inhibition of GH by insulin. In our study, although the CHD group was diagnosed as pre-diabetic, insulin levels in this group were found to be normal or slightly high. In CHD patients, in whom insulin synthesis is abnormal or absent, long-term insulin administration was used in the treatment of diabetes to take over the function of endogenous insulin. This is confirmed by the fact that the differences in GH, IGF-1 and IGFBP-3 levels between DM and CHD patient groups were statistically significant in our study.

The equal distribution of homo-/hetero-dimers relating to *GHR*/exon-3 polymorphism indicates an association between CHD or DM and *GHR*/exon-3 polymorphism. Though it is not significant, it was found that the frequency of the GHR-d3 allele (*GHR*-fl/d3+*GHR*-d3/d3) in the CHD group was higher than in *GHR*-fl/fl genotypic patients. Our results with GH, IGF-1 and IGFBP-3 levels in relation to variants of *GHR*/exon-3 polymorphism also revealed an alteration. Furthermore, blood glucose and IGF-1 levels were found to be lower and GH levels were higher in diabetics carrying the *GHR*-fl allele (*GHR*-fl/fl+GHR-fl/d3) than those with the *GHR*-d3/d3 genotype. GH levels in patients with GHR-fl allele were found to be high due to the fact that the biologic activity of *GHR*-fl allele-mediating metabolism and inactivation of GH is low.

The frequency of *GHR*-fl/fl genotype in men and *GHR*-d3 allele in women was found to be significantly high, a finding that may explain why the density of muscle mass is bigger than that in women. GH is an anabolic hormone responsible for the regulation of energy balance and, thus, may contribute to weight control as an alternative way androgens do in men. Because a biologically-active mutant form of GH is found in men, its metabolic effects, *e.g.* inhibition of protein oxidation, becomes more powerful and, as a result, causes muscle mass to be bigger in men than in women. In the CHD group with more active *GHR*-d3/d3 genotype, the probability of BMI≥27kg/m^2 was higher than in the group possessing the *GHR*-fl allele. This may also be due to variation in the biological activity of receptors.

In conclusion, hormonal control in establishing physiological states is of vital importance. The lines of evidence obtained in this study were as follows: i) GH, IGF-1, IGFBP-3 and insulin thought to act together to establish body homeostasis in patients with DM and CHD; ii) in diabetics, insulin–dependent inhibition of GH becomes weaker and this will, in turn, cause elevation of GH and IGF-1, which was acting like insulin on excess levels of glucose and, thus, works as a compensation mechanism against insulin deficiency; iii) in CHD, repair of tissue or organ damage is achieved by letting free the bound IGF-1 for protein synthesis; iv) in order to establish physiological glucose levels in CHD patients, GH release is either inhibited or kept close to controls; v) a direct effect of *GHR*/exon-3 polymorphism was observed in DM or CHD patients.

The limitation of our study is the size of study groups; therefore, the data obtained here should be supported by additional studies and further cell culture studies must be performed in order to prove our hypothesis.

Conflicts of Interest

The Authors declare that no competing interests exist.

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