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RESEARCH ARTICLE

The Relationship between Plasma Ghrelin Concentrations and Carotid Atherosclerosis in Type 2 Diabetes Patients

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Abstract

Background: Ghrelin, a peptide hormone which is secreted from the stomach and stimulates growth hormone release and had an orexgenic effect. Ghrelin had beneficial cardiovascular effect through its interaction with growth hormone secretagogue receptor (GHSR) which is widely distributed in the cardiovascular system and upregulated in atherosclerotic arteries but the association between ghrelin and carotid atherosclerosis had conflicting results in the previous studies.

Objective: To investigate the association between plasma ghrelin concentrations and carotid atherosclerosis, measured by carotid-intima media thickness (CIMT) in type 2 diabetes patients.

Study Design: This is an observational case-controlled study conducted in Ain Shams University hospital. The patient group included 50 patients with T2DM (25 obese and 25 lean patients). The control group included 36 healthy subjects (18 obese and 18 lean subjects). In both groups the fasting plasma ghrelin level, fasting plasma glucose, fasting serum insulin, HbA_{1C}, complete lipids profile, insulin resistance (HOMA-IR) and hs-CRP were measured. The degree of atherosclerosis was assessed by measuring the CIMT.

Results: Ghrelin was significantly lower in the diabetic obese patients than the diabetic non obese patient ($p<0.001$), also, it was lower in the non diabetic obese subjects than the non diabetic non obese subjects ($p<0.001$). BMI, WHR, FPG, HbA1c, fasting insulin, HOMA-IR, total cholesterol, triglycerides, LDL cholesterol, hs.CRP, and CIMT were significantly higher ($p<0.001$) in the diabetic obese patients. In the all studies subjects, ghrelin had statistical significant negative correlation with BMI, waist circumference, insulin, HOMA-IR, total cholesterol, LDL cholesterol, hs.CRP, CIMT ($p<0.001$), HbA1c, and triglycerides ($p<0.05$). In the diabetic obese patients, ghrelin had statistical significant negative correlation with BMI ($r= -0.4$, $p= 0.03$), CIMT ($r= -0.6$, $p= 0.01$), and HDL cholesterol ($r= -0.6$, $p=<0.001$). In the all studied populations CIMT had highly statistical significant positive correlation with all measured parameters ($p<0.001$), except with HDL cholesterol and ghrelin; there was highly statistical significant negative correlation ($p<0.001$).

Conclusion: ghrelin had significantly negative correlation with CIMT in the diabetic obese patients and it can be used a marker of subclinical atherosclerosis.

Abbreviations: body mass index (BMI), carotid-intima media thickness (CIMT)**,** fasting plasma glucose (FPG), growth hormone (GH), growth hormone secretagogue receptor (GHSR), high density lipoprotein (HDL), highly sensitivity CRP (hs-CRP), Homeostatic Model Assessment (HOMA), insulin resistance (IR), low density lipoprotein (LDL), type 2 diabetes mellitus (T2DM), waist/hip ratio (WHR),

Introduction

Ghrelin, a 28-amino acid peptide, secreted by ghrelin cells of gastric mucosa **[1]**, It is a natural ligand of the growth hormone secretagogue receptor (GHSR), which is widely distributed in the body, including the cardiovascular system **[2]**. In addition to its marked growth hormone (GH) releasing activity **[1]**, ghrelin exerts pleiotropic biological actions, prominently on the regulation of food intake, gastrointestinal motility, and energy homeostasis. In addition, there is a growing body of evidence that ghrelin is also involved in stress pathways **[3]**. Lastly, ghrelin seems to be involved in the modulation of cardiovascular, and immune functions **[4]**. Numerous studies have reported that ghrelin has a wide array of beneficial cardiovascular properties such as vasodilation **[5]** and beneficial hemodynamic effects in healthy humans **[6]** as well as improvement of left ventricular dysfunction and cardiac cachexia **[7]**. These effects are most probably GH-independent **[8]**. Low ghrelin concentrations have been associated with metabolic disturbances such as insulin resistance and type 2 diabetes mellitus (T2DM) **[9]**, which are commonly recognized as risk factors of atherosclerosis. Non-invasive measurements of surrogate markers of atherosclerosis, such as carotid-intima media thickness (CIMT), can be helpful in detecting subclinical diseases, especially among individuals at highest cardiovascular risk and determining the intensity of pharmaceutical treatment **[10]**. Beside conventional risk factors like dyslipidemia, hypertension, and hyperglycemia, C-reactive protein which is an acute phase reactant produced in the liver in response to adipocytokines, considered a strong predictor of cardiovascular diseases **[11]**. Few studies have investigated the association between ghrelin and CIMT as a marker of subclinical atherosclerosis and these studies had conflicting result **[12]**. In our study we hypothesized ghrelin to be associated with early carotid atherosclerosis, implicating an interaction between obesity and atherosclerosis in T2DM. To investigate this hypothesis, we measured plasma levels of ghrelin in diabetic patients and healthy subjects and we concomitantly assayed CIMT using ultrasound.

Subjects & Methods

We enrolled 86 subjects who were recruited from the Outpatient Clinic of Internal Medicine Departments, Ain Shams University Hospital during the period from July 2012 to June 2013. They were divided into 2 groups: **Group 1(case group)** which included 50 patients with T2DM (group 1a which included 25 obese patients (body mass index (BMI)>30kg/m^2) and group 1b which included 25 lean patients (BMI<25kg/m^2). **Group 2 (control group)** which included 36 healthy subjects who were age and sex matched and they were divided into group 2a which included 18 obese individuals and group 2b which included 18 lean individuals.

Inclusion criteria for selection of the patients were: type 2 diabetic patients of both sexes, their age 25-65 years, with onset of diabetes of less than 5 years, with normal hepatic and renal function, normotensive or hypertensive persons with blood pressure less than 130/80. We excluded patients with endocrinopathies, overt cardiovascular diseases, using lipid lowering drugs, using steroids, or subjects with BMI 25-29.9 Kg/m^2. Written informed consent was obtained from all participants including the purpose and procedures of the study. Approval had been taken from the research ethics committee, Faculty of Medicine, Ain Shams University.

All subjects of this study were submitted to full medical history, thorough clinical examination including the anthropometric measures [BMI and waist/hip ratio (WHR)], and fundus examination for the diabetic patients to exclude diabetic retinopathy. All measurements were carried out after a 12-h fast.

Blood samples were drawn from a peripheral vein after an overnight fast, between 8.00 and 10.00 am. Fasting plasma glucose (FPG) and lipids parameters were measured in mg/dl by an automatic enzymatic analyzer (Olympus AU560, Germany)**.** Fasting insulin level was measured in (µIU/ml) using commercial insulin kits of ELISA technique supplied by Biosource which was used as instructed by the manufacturer (catalog No. KAQ1251)**.** Assessment of insulin resistance (IR) index was done by using Homeostatic Model Assessment (HOMA). IR_{HOMA} = Fasting Insulin (µIU/ml) x FPG(mmol/L)/22.5 **[13].** HbA_{1C} level was measured by retaining haemoglobin by a cationic exchange resin (catalog No. U0897h).

Serum fasting Ghrelin level was measured in pg/dl by enzyme-linked immunosorbent assay (ELISA) method using the BioVendor Human Acylated Ghrelin ELISA diagnostic kit (Germany), samples were withdrawn in tubes containing EDTA and p hydroxymercuribenzoic acid to prevent the degradation of acylated ghrelin by protease.

Highly sensitivity CRP (hs-CRP) was measured in mg/l by immunoenzymometric assay**,** using The AccuBind ELISA Microwells diagnostic kit (USA) (product Code: 3125-500). Samples for (ghrelin, insulin, hs-CRP) were centrifuged and the separated serum was kept frozen & stored at - 20°C until ELISA analysis is duplicate.
Assessment of bilateral common carotid artery intimal medial thickness was done by high-resolution B-mode ultrasound using 7.5 MHZ linear array superficial probe. IMT was assessed at the bifurcation of the common carotid artery. The IMT was defined as the mean of the maximal value for the far walls on both the right and left sides. Thickness of more than1.05 mm is suggestive of atherosclerotic disease **[14]**.

Statistical methodology

Data was analyzed on an IBM personal computer using Statistical Package for Special Science (SPSS) software computer program version 18. Description of all data in the form of mean (M) and standard deviation (SD) for all quantitative variables was done. Frequency and percentage for all qualitative variables was calculated. Comparison between quantitative variables was done using t-test to compare two groups and ANOVA (analysis of variance) to compare more than two groups. Comparison of qualitative variables was done using the Chi-square test. Correlation coefficient also was done to find linear relation between different variables using r-test or Sperman correlation co efficient. Significant level measured according to P value (probability), P>0.05 is insignificant, and P<0.05 is significant.

Results

Clinical and biochemical characteristics of the whole study population, diabetic and non diabetic subjects (obese and non obese) are presented in **Table 1**. BMI, WHR, FPG, HbA1c, fasting insulin, HOMA-IR, total cholesterol, triglycerides, LDL cholesterol, hs.CRP, and CIMT were significantly higher (p<0.001) in the diabetic obese patients (**Table 1**). Ghrelin was significantly lower (p<0.001) in the diabetic obese patients **(Table 1& Figure 1)**. No significant difference was reported between the groups age and sex (**Table 1**),

As regard the correlation of ghrelin to different clinical and biochemical parameters, in the all studies subjects, ghrelin had statistical significant negative correlation with BMI, waist circumference, insulin, HOMA-IR, total cholesterol, LDL cholesterol, hs.CRP, CIMT (p<0.001)**,** HbA1c**,** and triglycerides (p<0.05) as shown in **Table 2**. There was insignificant correlation with age (p=0.8), WHR (p=0.3), FPG (p=0.1), and HDL cholesterol (p=0.5) **(Table 2)**.

In the diabetic obese patients, ghrelin had statistical significant negative correlation with BMI (r= -0.4, p= 0.03), CIMT (r= -0.6, p= 0.01) **(Table 3& Figures 2&3)**, and HDL cholesterol (r= -0.6, p=<0.001) **(Table 3)**. There was insignificant correlation with the other parameters **(Table 3)**.

In the all studied populations CIMT had highly statistical significant positive correlation with all measured parameters (p<0.001), except with HDL cholesterol and ghrelin; there was highly statistical significant negative correlation (p<0.001). There was an insignificant correlation with insulin **(Table 4)**. A forward stepwise regression analysis found that CIMT has only six predictors which are BMI, waist circumference, FPG, HbA1c, total cholesterol, and triglycerides **(Table 5)**.

Table (1): Baseline laboratory and clinical characteristics of the study participants

	(1a) Diabetic obese (n=25)	**(1b) Diabetic non obese (n=25)**	**(2a) Non diabetic obese (n=18)**	**(2b) Non diabetic non obese (n=18)**	**P**
Male no and%	14 (56%)	14 (56%)	10 (55.6%)	10 (55.6%)	>0.05
Female no and%	11 (44%)	11 (44%)	8 (44.4%)	8 (44.4%)	>0.05

Age(years)	44.6 ± 9.34	43.96 ± 8.43	43 ± 11.67	42.28 ± 13.09	>0.05
BMI(kg/m^2)	34.42 ± 3.18	24.58 ± 0.34	32.89 ± 1.70	23.88 ± 0.96	<0.001**
WHR	0.98 ± 0.08	0.97 ± 0.07	0.94 ± 0.07	0.87 ± 0.07	<0.001**
FPG(mg/dl)	171.40 ± 61.26	162.72 ± 66.98	95.83 ± 14.52	90.39 ± 15.70	<0.001**
HbA1c (%)	8.70 ± 1.65	8.46 ± 2.05	5.97 ± 0.50	5.49 ± 0.64	<0.001**
insulin (miu/ml)	17.85 ± 5.79	11.96 ± 6.14	17.71 ± 3.18	14.02 ± 2.95	<0.001**
HOMA-IR	7.50 ± 3.65	4.00 ± 1.03	4.15 ± 0.80	3.14 ± 0.89	<0.001**
Total cholesterol (mg/dl)	226.36 ± 52.51	212.04 ± 42.52	212.33 ± 22.44	156.67 ± 22.33	<0.001**
Triglycerides (mg/dl)	185.24 ± 66.42	178.84 ± 80.07	155.17 ± 21.13	97.22 ± 25.40	<0.001**
LDL cholesterol (mg/dl)	146.48 ± 45.63	139.04 ± 33.84	139.67 ± 23.45	87.83 ± 24.30	<0.001**
HDL cholesterol (mg/dl)	41.88 ± 10.26	39.40 ± 5.79	41.56 ± 7.21	49.56 ± 7.81	<0.001**
Ghrelin (pg/ml)	113.38 ± 21.82	169.50 ± 23.90	125.33 ± 48.63	208.17 ± 34.07	<0.001**
hs.CRP (mg/l)	4.47 ± 1.62	2.53 ± 0.71	2.51 ± 0.78	0.83 ± 0.21	<0.001**
CIMT (mm)	1.09 ± 0.20	0.98 ± 0.15	0.82 ± 0.08	0.72 ± 0.09	<0.001**

Data are expressed as frequency and percentage data. Data are expressed as mean ± SD for parametric data. Comparisons between four groups done using ANOVA, $p<0.05$ *, $p<0.001$ **. Ghrelin level was the highest among group (2b) the non diabetic non obese subjects and the lowest level was among group (1a) the diabetic obese patients. hs.CRP and CIMT levels were the highest among group (1a) and were the lowest among group (2b).

Table (2): Correlation of ghrelin to different clinical and biochemical parameters in all studied individuals

	Ghrelin		
	Pearson correlation coefficient	**p**	**Sig.**
Age (yr)	0.025	0.819	NS
BMI (Kg/m^2)	-0.721	<0.001	HS

Waist circumference (cm)	-0.449	<0.001	HS
WHR	-0.101	0.354	NS
FPG (mg/dL)	-0.151	0.166	NS
Insulin (MIU/L)	-0.354	<0.001	HS
HOMA-IR	-0.398	<0.001	HS
HbA1c (%)	-0.235	0.029	S
Total cholesterol (mg/dL)	-0.364	<0.001	HS
Triglycerides (mg/dL)	-0.235	0.029	S
LDL cholesterol (mg/dL)	-0.343	<0.001	HS
HDL cholesterol (mg/dL)	0.071	0.517	NS
hs.CRP (mg/L)	-0.537	<0.001	HS
CIMT (mm)	-0.486	<0.001	HS

In all studied individuals, ghrelin had highly statistical significant negative correlation with BMI, waist circumference, insulin, HOMA-IR, total cholesterol, LDL cholesterol, hs.CRP, and CIMT and statistically significant negative correlation with HbA1c and triglycerides. There was insignificant correlation with age, WHR, FPG, and HDL cholesterol.

r- Pearson correlation. P-value <0.001 HS ; p-value <0.05 S ; p-value >0.05 NS

Table (3): Correlation of ghrelin to different clinical and biochemical parameters in diabetic obese patients (group 1a)

	Ghrelin		
	Pearson correlation coefficient	**P**	**Sig.**
Age (yr)	0.150	0.475	NS
BMI (Kg/m^2)	-0.418	0.037	S
Waist circumference(cm)	0.236	0.158	NS

WHR	0.107	0.550	NS
FPG (mg/dL)	0.077	0.713	NS
Insulin (MIU/L)	0.180	0.388	NS
HOMA-IR	0.165	0.430	NS
HbA1c (%)	-0.056	0.789	NS
Total cholesterol (mg/dL)	-0.094	0.655	NS
Triglycerides (mg/dL)	0.078	0.710	NS
LDL cholesterol (mg/dL)	0.017	0.936	NS
HDL cholesterol (mg/dL)	-0.635	<0.001	HS
hs.CRP (mg/L)	-0.118	0.575	NS
CIMT (mm)	-0.695	0.018	S

In the diabetic obese patients (group 1a) ghrelin had statistically significant negative correlation with BMI and CIMT. There was highly statistical significant negative correlation with HDL cholesterol. There was insignificant correlation with the other parameters.

r- Pearson correlation. P-value <0.001 HS ; p-value <0.05 S ; p-value >0.05 NS.

Table (4): Correlation of CIMT to different clinical and biochemical parameters in all studied groups

	CIMT		
	Pearson correlation coefficient	**p**	**Sig.**
Age (yr)	0.540	<0.001	HS
BMI (Kg/m^2)	0.379	<0.001	HS
Waist circumference (cm)	0.392	<0.001	HS

WHR	0.339	0.001	HS
FPG (mg/dL)	0.644	<0.001	HS
Insulin (MIU/L)	0.055	0.617	NS
HOMA-IR	0.555	<0.001	HS
HbA1c (%)	0.684	<0.001	HS
Total cholesterol (mg/dL)	0.548	<0.001	HS
Triglycerides (mg/dL)	0.624	<0.001	HS
LDL cholesterol (mg/dL)	0.514	<0.001	HS
HDL cholesterol (mg/dL)	-0.447	<0.001	HS
Ghrelin (Pg/dL)	-0.486	<0.001	HS
hs.CRP (Mg/L)	0.697	<0.001	HS

In the all studied groups CIMT had highly statistical significant positive correlation with all measured parameters, except with HDL cholesterol and ghrelin, there was highly statistical significant negative correlation. There was an insignificant correlation with insulin.

r- Pearson correlation. P-value <0.001 HS ; p-value <0.05 S ; p-value >0.05 NS.

Table (5): Forward Stepwise Regression Analysis for determination of the independent factors of CIMT:

Independent variable	**Coefficient (B)**	**t**	**P**
(Constant)	-0.210	-0.756	0.034
BMI (kg/m2)	0.008	0.696	0.049
FPG (mg/dl)	0.010	0.784	0.038
HbA1c (%)	0.074	0.628	0.042
Total cholesterol (mg/dl)	-0.018	-0.939	0.037
Triglycerides (mg/dl)	0.003	0.576	0.045
Waist circumference (cm)	-0.003	-0.898	0.023

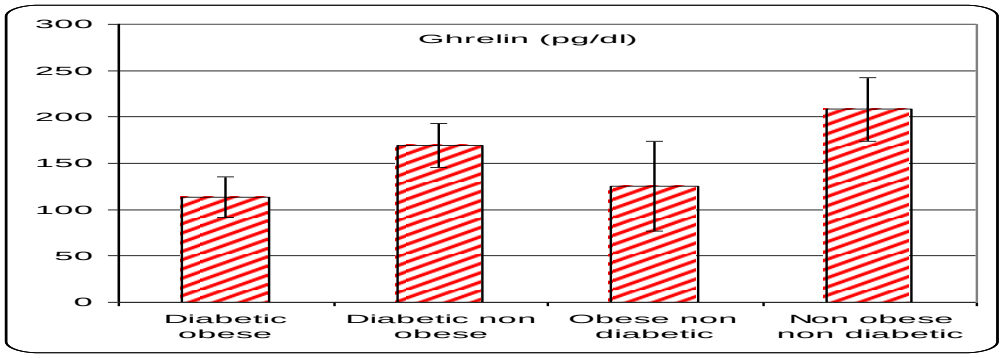

Figure (1) shows comparison between the studied groups as regard the mean value of serum ghrelin (pg/dl) with the highest level was among the non obese non diabetic subjects and the lowest level was among the diabetic obese patients.

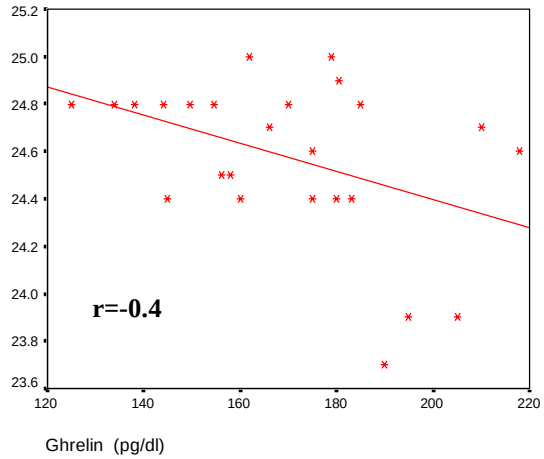

Figure (2) shows the statistically negative correlation between ghrelin and BMI in the diabetic obese patients (group 1a) (p=0.03, r=-0.4).

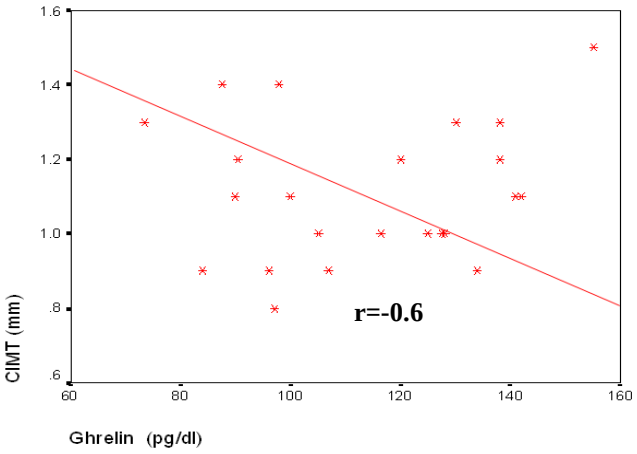

Figure (2) shows the statistically negative correlation between ghrelin and CIMT in the diabetic obese patients (group 1a) (p=0.01, r=-0.6).

Discussion

In the present cross-sectional study we demonstrated significantly lower plasma levels of ghrelin in the diabetic obese patients compared with age- and sex matched healthy individuals. Low ghrelin levels in obese diabetic patients were reported earlier by **Tschöp and colleagues [15]**. Although weight gain and obesity were not the focus

of our study, we assessed the effect of ghrelin on weight gain, because ghrelin is closely associated to energy homeostasis, weight regulation and obesity. Obesity is one of the risk factors for atherosclerosis and it is becoming a more and more serious health problem all around the world. The plasma ghrelin level was inversely associated with BMI in the present study, this correlation was highly significant in the all studied population ($r=-0.7$, $p<0.001$), also, it was highly significant among the control group ($r=-0.8$, $p<0.001$), and it was significant in the diabetic obese group ($r=-0.4$, $p=0.03$). Circulating plasma ghrelin levels are reportedly inversely associated with BMI in humans **[16]**. In humans, ghrelin secretion is decreased in obesity and is normalized following recovery to ideal body weight **[15]**. Thus, the associations seen between plasma ghrelin levels and BMI in this study are consistent with the previously reported findings.

To our knowledge there were few studies investigated the relation between plasma ghrelin levels and carotid atherosclerosis and they showed conflicting results, We found that ghrelin had highly significant negative correlation with CIMT in the all studied individuals ($r=-0.4$, $p<0.001$) and significant negative correlation in the diabetic obese group ($r=-0.6$, $p=0.018$), in the other studied groups the correlation between ghrelin and CIMT was negative but statistically insignificant, this finding may be due to decrease sample size in the other subgroups and the statistical effect of the diabetic obese group on the all studied population ($r=-0.695$ in the diabetic obese group, $r=-0.486$ in the all studied individuals).

Our results is similar to a study on 101 older Japanese patients >60 years old (mean age 72.3) with metabolic syndrome that ghrelin had significant negative correlation with CIMT, and in the multiple regression analysis for CIMT adjusted by other measured parameters, ghrelin was significant and independent factor along with age and systolic blood pressure **[17]**. In the study of **Yano and colleagues [18]**, they were found similar results to **Kotani and colleagues [17]** and our study, they found in a study on 263 elderly hypertensive patients (mean age 72.6) that plasma des-acyl ghrelin had significant inverse correlation with CIMT ($p=0.003$) and the multivariable regression analysis showed that des-acyl ghrelin was significantly associated with CIMT after adjusting for age, obesity, sex, smoking, 24 hours blood pressure, and other cardiometabolic factors. Also, **Kadoglou and colleagues [19]** found significant inverse correlation between ghrelin and CIMT in 124 patients with type 2 diabetes (mean age 65.09) independent of other metabolic parameters ($r = -0.4$, $p = 0.002$).

Furthermore, in a study of kidney transplantation patients there was no association between the plasma ghrelin level and CIMT **[20]**. Also, **Hajmohammadi and colledagues** had been demonstrated that ghrelin had no significant correlation with CIMT in the metabolic syndrome patients **[12]**.

Opposite results were found by others, **Pöykkö and colleagues [21]** discovered that plasma ghrelin concentrations associated positively with the degree of subclinical atherosclerosis measured as CIMT. In the analysis of whole data set the association was of borderline significance, whereas in the subgroup analysis the association between CIMT and ghrelin concentrations was observed only in males. Also, in another study of **Ukkola and colleagues [22]**, they had found that ghrelin had positive association with CIMT ($p<0.005$) but the independent positive association ($p<0.01$) between the plasma ghrelin quartile and CIMT was evident in the lowest IGF-1 quartile only, they concluded their finding that ghrelin-leptin relation is associated with early atherosclerosis and the interaction between IGF-1 and ghrelin modifies the association of ghrelin with early atherosclerosis.

Previous studies have shown positive **[21, 22]**, negative **[17, 18, 19]**, or no relationship **[12, 20]** of serum ghrelin levels with CIMT, the above conflicting results may be attributed to the great variation in the studies population characteristics and statistical methods. Another explanation is that other metabolic factors, such as leptin, IGF-I, etc., might have affected ghrelin levels in the aforementioned studies.

Most reports suggest a beneficial role for ghrelin in normal and pathological conditions of the cardiovascular system. Both ghrelin and the ghrelin receptor have been shown to be expressed in cardiovascular system **[23]**, the density of the GHSR, the target of ghrelin, has been shown to be upregulated in atherosclerotic carotid arteries, reflecting the counter regulatory role of ghrelin in atherosclerosis **[24]**. Endothelial dysfunction is considered to be one of the earliest events of the atherosclerotic development. In patients with endothelial dysfunction, plasma ghrelin level decreases **[25]**, conversely, the application of exogenous ghrelin can improve endothelial dysfunction in metabolic syndrome patients, suggesting that high ghrelin levels might be beneficial for endothelial function **[26]**.

From the present study we conclude that plasma ghrelin is negatively associated with CIMT, and it can be used as a marker of subclinical atherosclerosis. We suggest further studies on the precise mechanism by which ghrelin regulates atherosclerosis that may provide key insights into ghrelin's administration in atherosclerosis therapy.

References:

1. **Kojima M, Hosoda H, Date Y, et al.** Ghrelin is a growth-hormone releasing acylated peptide from stomach. Nature. 1999; 402: 656-660.

2. **Gnanapavan S, Kola B, Bustin S, et al.** The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. Journal of Clinical Endocrinology and Metabolism. 2002; 87: 2988.

3. **Stengel A, Wang L, Taché Y**. Stress-related alterations of acyl and desacyl ghrelin circulating levels: mechanisms and functional implications. Peptides. 2011; 32: 2208–2217.

4. **Ukkola O**. Ghrelin in Type 2 diabetes mellitus and metabolic syndrome. Mol. Cell. Endocrinol. 2011; 340: 26–28.

5. **Nagaya N, Kojima M, Uematsu M, et al.** Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2001a; 280: 1483-1487.

6. **Nagaya N, Uematsu M, Kojima M, et al.** Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation. 2001b; 104: 1430-1435.

7. **Nagaya N, Kangawa K.** Ghrelin improves left ventricular dysfunction and cardiac cachexia in heart failure. Curr Opin Pharmacol. 2003a; 3 (2): 146-151.

8. **Isgaard J, Barlind A, Johansson I.** Cardiovascular effects of ghrelin and growth hormone secretagogues. Cardiovasc Hematol Disord Drug Targets. 2008; 8 (2): 133-137.

9. **Pöykkö SM, Kellokoski E, Hörkkö S, et al.** Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes. 2003a; 52(10): 2546–2553.

10. **Kathiresan S, Larson MG, Keyes MJ, et al**. Assessment by cardiovascular magnetic resonance, electron beam computed tomography, and carotid ultrasonography of the distribution of subclinical atherosclerosis across Framingham risk strata. Am J Cardiol. 2007; 99: 310-314.

11. **Bastard JP, Maachi M, Lagathu C, et al**. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2006; 17: 4-12.

12. **Hajmohammadi Y, Sadeghi M, Dashti M, et al**. Relationship between carotid intima-media thickness with some inflammatory biomarkers, ghrelin and adiponectin in Iranian with and without metabolic syndrome in Isfahan cohort study. ARYA Atherosclerosis Journal. 2010; 6(2): 56-61.

13. **Matthews, DJ, Hoskers J.P, Rudenski A.S, Waylur B.A, Trencher D.Y and Turner R.C.** Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.

14. **Feinstein SB.** The powerful microbubble: from bench to bedside, from intravascular indicator to therapeutic delivery system, and beyond. Am J Physiol Heart Circ Physiol. 2004; 287 (2): 450-457.

15. **Tschöp M, Weyer C, Tataranni PA, et al.** Circulating ghrelin levels are decreased in human obesity. Diabetes. 2001b; 50 (4): 707-709.

16. **Cummings DE**. Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav. 2006; 89(1): 71–84.

17. **Kotani K, Sakane N, Saiga K, et al.** Serum ghrelin and carotid atherosclerosis in older Japanese people with metabolic syndrome. Arch Med Res. 2006; 37(7): 903–906.

18. **Yano Y, Toshinai K, Inokuchi T, et al.** Plasma des-acyl ghrelin, but not plasma HMW adiponectin, is a useful cardiometabolic marker for predicting atherosclerosis in elderly hypertensive patients. Atherosclerosis. 2008; 204(2): 590-594.

19. **Kadoglou N, Sailer N, Moumtzouoglou A, et al**. Visfatin (nampt) and ghrelin as novel markers of carotid atherosclerosis in patients with type 2 diabetes. Exp Clin Endocrinol Diabetes 2010; 118: 75-80.

20. **Genis BB, Granada ML, Alonso N, et al.** Ghrelin, glucose homeostasis, and carotid intima media thickness in kidney transplantation. Transplantation. 2007; 84 (10): 1248-1254.

21. **Pöykkö SM, Kellokoski E, Ukkola O, et al**. Plasma ghrelin concentrations are positively associated with carotid artery atherosclerosis in males. J Intern Med. 2006; 260: 43-52.

22. **Ukkola O, Poykko S, Paivansalo M et al**. Interactions between ghrelin, leptin and IGF-I affect metabolic syndrome and early atherosclerosis. Ann Med 2008; 40: 465-473.

23. **Kleinz M, Maguire J, Skepper J, Davenport A.** Functional and immunocytochemical evidence for a role of ghrelin and desoctanoyl ghrelin in the regulation of vascular tone in man. Cardiovasc Res. 2006; 69: 227-235.

24. **Katugampola SD, Pallikaros Z, Davenport AP.** [125I-His(9)]-ghrelin, a novel radioligand for localizing GHS orphan receptors in human and rat tissue: upregulation of receptors with atherosclerosis. Br J Pharmacol. 2001; 134 (1): 143-149.

25. **Tajtakova M, Petrovicova J, Spurny P, et al.** Selected hormones levels in individuals with endothelial dysfunction and insulin resistance. Bratisl Lek Listy. 2005; 106: 37-40.

26. **Tesauro M, Schinzari F, Iantorno M**. Ghrelin improves endothelial function in patients with metabolic syndrome. Circulation. 2005; 112: 2986-2992.

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