# GHRELIN AS MARKER OF AGE ASSOCIATED CARDIO-VASCULAR DISEASE

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**Abstract**

Studies regarding the pathology associated with obesity are abundant in literature, but there is no clear opinion regarding the relationship between the cardio-vascular risk and the age of patients. Several studies are nowadays devoted to finding the parameters that would allow a precise evaluation of the health status of ageing patients. Cardio-vascular diseases (CVD) represent the main co-morbidity of the ageing process.

The aim of the present study was the assessment of ghrelin dynamics in serum samples obtained from patients with different age, with or without cardio-vascular diseases. Our results show a negative association between the age of the selected patients and the ghrelin level in blood samples. Also, the cardio-vascular patients were characterized by a lower level of ghrelin compared to healthy subjects. Results lead to the conclusion that ghrelin is a reliable marker for the cardio-vascular risk and that ghrelin modulation should be considered as a novel therapeutic strategy.

**Rezumat**

Studiile care se adresează patologiei asociate cu obezitatea sunt numeroase în literatura de specialitate, dar nu există teorii clare şi general acceptate în ceea ce priveşte relaţia dintre riscul cardio-vascular şi vârstă. În prezent se află în desfăşurare numeroase studii care au ca scop identificarea unor biomarkeri care să permită evaluarea cât mai precisă a stării de sănătate a pacienţilor vârstnici.

Scopul prezentului studiu a fost evaluarea dinamicii grelinei în probe de ser obţinute de la pacienţi cu vârste diferite, cu sau fără diagnostic de boală cardio-vasculară. Rezultatele au demonstrat existenţa unei corelaţii negative între nivelul de grelină şi vârstă, la pacienţii selectaţi. De asemenea, pacienţii diagnosticaţi cu boală cardio-vasculară sunt caracterizaţi prin niveluri semnificativ reduse ale grelinei comparativ cu subiecţii sănătoşi clinic. Rezultatele obţinute conduc la concluzia că grelina poate constitui un marker al afecţiunilor cardio-vasculare şi că modularea nivelului plasmatic al grelinei poate reprezenta o strategie terapeutică.

**Keywords:** ageing, ghrelin, cardio-vascular disease.

## Introduction

Ghrelin is a polypeptide secreted mainly by the stomach, that regulates homeostatic food intake, hedonic eating, and is a mediator in the stress response. The molecule is composed of 28 amino acids and the n-

octanoylation of serine 3 position in the molecule is necessary for the bioactivity. In addition, ghrelin has metabolic, cardiovascular, and anti-aging effects, is associated with metabolic and cardiovascular health, and may have anti-aging effects, but these effects may be attenuated in obesity. Total plasma ghrelin is decreased in obesity, providing evidence consistent with the theory that central resistance to ghrelin develops in obesity and ghrelin's function in appetite regulation may have evolved to prevent starvation in food scarcity rather than cope with modern food excess [1, 2].

Dyslipidaemia is characterised by a high free fatty acids level, an imbalance of leptin, resistin, and adiponectin and a deficiency of ghrelin. Literature data on the important role of ghrelin in glucose and lipid metabolism as well as energy homeostasis regulation suggest that ghrelin plays a role in insulin resistance development. Ghrelin was shown to contribute to the expression of α- and β-insulin receptor subunits. Obese children have lower ghrelin levels compared to those with normal metabolism [3].

Recent literature data suggest that the serum levels of ghrelin are significantly positively correlated with angiotensin II in chronic heart failure patients and that the peptide can play a role in preventing heart disease, being a new biomarker of chronic heart failure severity as well as a new prognostic predictor for this disease [4, 5].

Aging is associated with a decrease in appetite, energy intake and glucose tolerance. Experimental studies have suggested that ghrelin plays a role in glucose homeostasis and in the regulation of energy metabolism especially associated with age [6, 7]. Ghrelin's hallmark functions are its stimulatory effects on growth hormone release, food intake and fat deposition, constitutes an orexigenic signal, and also plays crucial roles in cardioprotection, muscle atrophy and bone metabolism [8].

The purpose of the present study was to assess the ghrelin concentrations for adults with or without cardio-vascular disease and to examine the role of ghrelin as a cardio-vascular marker on the selected groups.

**Materials and Methods**

*Subjects and study design*
The study comprised 50 subjects, aged 50–65 years, selected from the Humanitas Medical Center, Bucharest. All subjects recruited for the study were non-smokers. None of the subjects presented renal, hepatic, gastrointestinal, pulmonary, endocrine or oncological diseases or were receiving antioxidative vitamin supplementation for at least 6 weeks before

the study. The study was carried out according to the principles of the Declaration of Helsinki; all participants gave their informed consent; the Ethics Committee of Humanitas Medical Center has approved the study. Subjects were divided into two groups, considering the cardio-vascular disease diagnosis: cardio-vascular disease patients (n=29) and age matched controls (n=21). The diagnosis of cardio-vascular disease was based on clinical evaluation.

*Biochemical evaluation*

Blood samples were collected from all patients after overnight fasting (12 h) and were analysed for fasting plasma glucose, total cholesterol (TC), LDL-cholesterol, HDL-cholesterol, triglycerides (TG) (using Merck and Randox commercial kits).

For the assessment of active (acylated ghrelin) in blood samples an ELISA non-radioactive specific method was used (DRG Human Ghrelin). According to the manufacturer's indications, the active ghrelin molecule is extremely unstable in serum/plasma; for this purpose all samples were kept on ice all along the processing and were exposed to acidification (HCl 0.05 N) , that induced protein precipitation. Subsequently, the samples were centrifuged 3,000 x g for 15 minutes at $4 \pm 2°C$ and the supernatant was used for the ELISA assay.

*Statistical analyses*

Data are presented as mean ± SD. Clinical characteristics were compared using the *t Student* test. Significance was defined at the 0.05 level of confidence. All calculations were performed using the Statistical Package for Social Sciences software (SPSS).

### Results and Discussion

The biochemical parameters of the patients are presented in Table 1; the two groups are age matched (50-65 years old).

**Table I**

Biochemical parameters for the study groups

| Biochemical parameter | Control group | Cardio-vascular group |
|---|---|---|
| Total cholesterol (mg/dL) | 198.55±49.62 | 218.95±32.27* |
| LDL-chlesterol (mg/dL) | 122.45±44.60 | 136.90±26.19 |
| HDL-cholesterol (mg/dL) | 51.22±13.22 | 48.41±6.83 |
| Triglycerides (mg/dL) | 128.30±61.47 | 163.05±80.87 |
| Fasting plasma glucose (mg/dL) | 84.82±9.66 | 96.86±16.79 |
| Ghrelin (pg/mL) | 45.59±35.43 | 17.02±10.45* |

*p<0.05

The statistical analysis of the data pointed out that the cardio-vascular patients are characterized by significantly higher levels of the total

cholesterol, but all the other biochemical parameters were similar for the two study groups. This can be interpreted as a partial significance of the general metabolic profile in the evaluation of cardio-vascular risk for the selected patients. The assessment of the cardio-vascular risk cannot be performed only based on the level of lipid markers (TC, LDL and HDL).

The ghrelin level was significantly lower for the cardio-vascular group compared to the age matched controls. This difference suggests the involvement of ghrelin in the protection of cardio-vascular function. The results show that the ghrelin level might represent a useful marker in the assessment of cardio-vascular risk. Our results are in agreement with data from literature, stating that in a 8 years follow-up study, including metabolic syndrome patients, the ghrelin level was higher in patients characterized by lower abdominal perimeter, so in patients with lower risk associated with insulin resistance [9].

The multiples regression analysis revealed a negative, significant correlation between the age of the patients and the ghrelin level (p=0.05, r= -0.367) (Figure 1). This correlation was obtained when analysing the data for the whole group of patients.
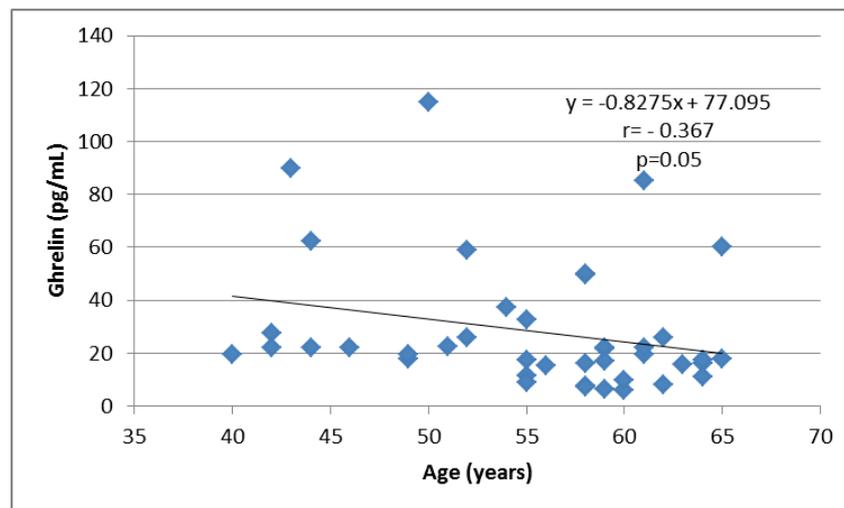


**Figure 1**
The correlation between the age and the ghrelin level for all the patients included in the study

The ghrelin level was also associated in a negative manner with the weight of the selected patients; the increase of the body weight is correlated with a decrease of the ghrelin level (Figure 2).
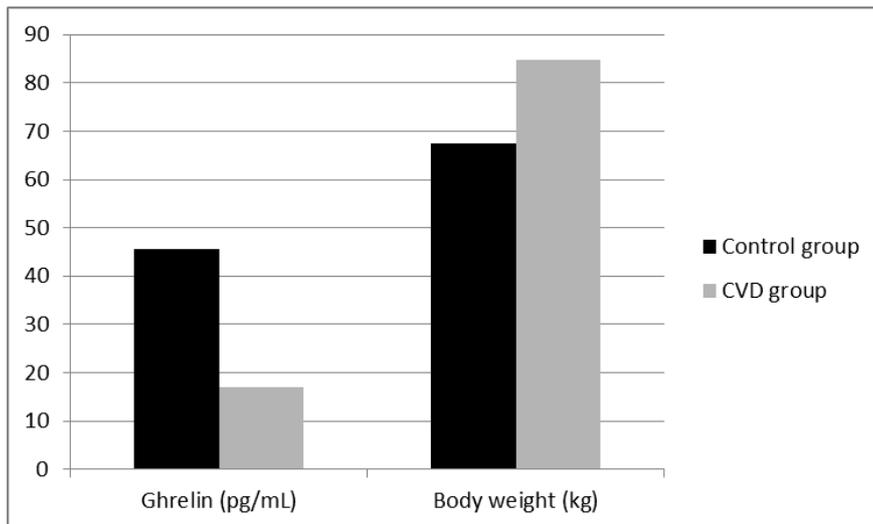
**Figure 2**
The dynamics of the ghrelin in correlation with the body weight for the two groups

According to literature data, obesity dramatically increases the risk of development of cardiovascular and metabolic diseases, which have their origins in endothelial dysfunction. Obesity-induced endothelial dysfunction is associated with decreased nitric oxide (NO) production due to impaired endothelial NO synthase activity and increased production of superoxide anion [10, 11, 12]. These results should be interpreted in view of experimental studies, performed locally, on the gastric mucosa, proving that ghrelin counters the proinflammatory consequences of the lipopolysaccharides, including the excessive generation of NO [13]. Studies performed on human endothelial cells, show that ghrelin decreases the reactive oxygen species in HUVECs and increased eNOS expression [14]. So, there is a negative association between ghrelin and NO, both locally, at the gastric level as well as at the level of endothelial cells, and in the general blood circulation.

**Conclusions**

The aim of the present study was to assess the ghrelin concentrations for adults with or without cardio-vascular disease and to examine the role of ghrelin as a cardio-vascular marker the selected groups. For this purpose, we selected 50 patients, aged 50 to 65 years, divided into two groups - cardio-vascular disease patients (n=29) and age matched healthy subjects constituting the control group (n=21). Our results show a negative association between the age of the selected patients and the ghrelin level in blood samples; so the increase of the age is associated with the decrease of

the ghrelin level, thus pointing out the role of ghrelin in the ageing process. We also evidenced the protective role of ghrelin by report to the cardio-vascular function; the increase of the body weight of the patients was associated with a decrease of the ghrelin level. Also, the cardio-vascular patients were characterized by a lower level of ghrelin compared to healthy subject.

Results lead to the conclusion that ghrelin is a reliable marker for the cardio-vascular risk, the disadvantage being represented by the necessity of rather complicated routine in the preparative phase of the sample evaluation. Also, ghrelin modulation should be considered as a novel therapeutic strategy; pharmacological and dietetic methods leading to the increase of the ghrelin level might constitute important tools for the reduction of cardio-vascular risk, especially in elderly patients.

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