MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2024; 30: e944462
DOI: 10.12659/MSM.944462

Received: 2024.03.12
Accepted: 2024.05.07
Available online: 2024.06.06
Published: 2024.06.27

Elevated Plasma Levels of Growth Arrest Specific 6 (Gas6) Protein in Severe Obesity: Implications for Adipose Tissue and Inflammation

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Financial support: This work was supported by the Italian Ministry of Health (Ricerca Corrente 18C203_2012)
Conflict of interest: None declared

Background: Preliminary data suggest an adipogenic role for growth arrest-specific 6 (Gas6), a pleiotropic molecule involved in inflammation, proliferation, and hemostasis through its Tyro3, Axl, and MerTK (TAM) receptors. This study compares Gas6 expression in plasma and visceral and subcutaneous adipose tissue in 42 adults with obesity (body mass index ≥40 kg/m^2) and 32 normal-weight controls to elucidate its role in obesity and related metabolic alterations.

Material/Methods: Using a case-control design, we measured Gas6 levels in plasma via a validated sandwich enzyme-linked immunosorbent assay and in adipose tissues through quantitative polymerase chain reactio with specific probes. Medians and correlations were analyzed using Mann-Whitney and Spearman tests. A general linear model assessed the impact of covariates on the Gas6-anthropometric relationship, with statistical significance determined by *P* values.

Results: Plasma Gas6 levels were significantly higher in the obese group than in controls (*P*=0.0006). While Gas6 mRNA expression did not significantly differ in subcutaneous adipose tissue between groups, it was notably higher in visceral than subcutaneous adipose tissue in controls (*P*<0.05). A significant correlation was found between plasma Gas6 levels and body mass index (*P*=0.001).

Conclusions: Gas6 plasma levels are elevated in morbid obesity, particularly in visceral adipose tissue, and are linked to altered glucose tolerance in female patients. These findings highlight the role of Gas6 in obesity-related metabolic complications and suggest avenues for further research and potential therapies.

Keywords: FTO Protein, Human • Abdominal Obesity Metabolic Syndrome • Obesity • Growth Arrest-Specific Protein 6 • Adipose Tissue • Intra-Abdominal Fat

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/944462

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Introduction

Adipose tissue, traditionally viewed as an inert energy storage depot, has emerged as a highly dynamic and metabolically active organ with profound implications for whole-body homeostasis [1]. Growth arrest-specific 6 (Gas6) is a vitamin K-dependent protein expressed in different tissues, including the gut, bone marrow, endothelial cells, and fibroblasts [2,3]. Gas6 has been identified as a ligand for 3 tyrosine-kinase receptors, Tyro3, Axl, and Mer, collectively named TAM [4]. TAM receptors are variably expressed in many tissues and can be found as a soluble form in the bloodstream as sTyro3, sAxl, and sMer, respectively [5]. Gas6 has been identified as a multifaceted mediator in various physiological processes, ranging from cell survival and proliferation to inflammation and immune regulation [6,7]. Gas6 utilizes a unique mechanism involving its vitamin K-dependent GLA module for binding phosphatidylserine-containing membranes and its LamG domains for interaction with TAM receptors. This dual interaction modulates cellular functions and signaling pathways crucial for leukocyte responses and homeostasis [8]. While the role of Gas6 and its receptors in immune modulation has been extensively studied, their involvement in adipose tissue physiology, metabolism, and obesity is an exciting and relatively unexplored avenue; preliminary preclinical studies are, however, promising. Experiments with mice fed a high-fat diet indicated that the upregulation of Gas6/TAM signaling might enhance adipogenesis and body fat accumulation [9]. The transmembrane receptor Axl is overexpressed in subcutaneous adipose tissue of obese human subjects compared with lean controls and might mediate Gas6 adipogenic activity [10]. Indeed, mice overexpressing Axl are characterized by an increased body weight, and, in a mouse model, the pharmacological and genetic inhibition of Axl increases the thermogenic energy expenditure of white and brown adipose tissue [11]; moreover, the inhibition of Axl impairs adipose tissue accumulation by downregulating preadipocytes differentiation into mature adipocytes [12], although a subsequent study demonstrated that the deletion of Axl does not determine differences in the development of adipose tissue in mice, since its role would be taken over by Mer and Tyro3 [13]. A 2021 study found that during adipocyte differentiation, Gas6 expression gradually decreases, particularly in the presence of inflammation and insulin resistance in adipose tissues. Furthermore, Gas6 levels were directly correlated with the expression of adiponectin, a key adipocytokine involved in improving insulin sensitivity. These findings indicate that Gas6 potentially modulates adiponectin levels, influencing metabolic processes linked to insulin resistance in adipocytes [14].

These observations, taken together, suggest that Gas6 can participate in adipogenesis by regulating cell proliferation and differentiation through TAM receptors and can subsequently affect the development of obesity. Consistently, higher levels of circulating Gas6 and sAxl have been demonstrated in overweight and obese adolescents than in lean controls, with circulating Gas6 levels significantly correlated with body mass index (BMI), waist circumference, waist-to-hip circumference ratio, and body fat mass [15,16].

It should also be acknowledged that the Gas6/TAM system is involved in the regulation of inflammatory response [17,18], tissue repair, fibrosis development [19,20], and vascular integrity [21,22], which are all crucial pathogenetic mechanisms of obesity complications. Thus, it is reasonable to postulate a role for this pathway not only in adipogenesis but also in the development of the complications that often parallel morbid obesity.

The present study aims to elucidate the association between Gas6 and obesity, also focusing on its effect on different comorbid conditions associated with overweight. Therefore, we aimed to compare Gas6 protein levels in plasma and visceral and subcutaneous adipose tissue in 42 adults with obesity (BMI ≥40 kg/m^2) and 32 controls with a normal body weight.

Material and Methods

Study Design and Compliance

We conducted a case-control study in strict adherence to the Declaration of Helsinki. The study protocol was approved by the local Ethics Committee in Milan, Italy, at its meeting on October 20, 2020 (ISTOFATOB, code: 2020_10_20_05), ensuring that all participants provided written informed consent. Informed consent complied with the standards for scientific studies and included a detailed and understandable summary of the study, purpose of the work, privacy criteria, times and methods of conservation of the biological material, and sensitive information of the participants.

Participant Recruitment

Cases were recruited among patients with severe obesity, referring to the IRCCS Istituto Auxologico Italiano (U.O. General Medicine, San Giuseppe Hospital, Oggebbio, Italy) for workup and rehabilitation of their obese status. We included 42 patients with a BMI ≥40 kg/m^2. We applied the following exclusion criteria: (1) previous diagnosis of diabetes mellitus, either type 1 or type 2; (2) previous diagnosis of coronary artery disease, chronic kidney disease, or chronic liver disease; (3) concomitant treatment with steroids or statins; (4) psychiatric disorders; and (5) pregnancy.

A total of 32 normal weight controls were recruited among patients undergoing non-neoplastic and non-inflammatory

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abdominal surgery at the General Surgery Department of the Maggiore della Carità Hospital in Novara.

Data Collection Upon Enrollment

Upon enrollment, the following data were collected: height, weight, BMI, waist and hip circumferences, waist-to-hip ratio, personal and family history, and current therapies. The waist was measured halfway between the costal edge and the crista. The hip was measured as the greatest circumference around the nates.

Routine laboratory data included a complete blood cell count, evaluation of renal and liver function tests, and C-reactive protein. Glucose metabolism was assessed by fasting plasma glucose and insulin levels during the oral glucose tolerance test and glycated hemoglobin (HbA1c) using current guidelines (ADA Standards of Medical Care in Diabetes 2019) [23]. Insulin resistance was calculated by the homeostatic model of insulin resistance (HOMA-IR) as fasting insulin (μU/m×[fasting PG (mmol/L)/22.5]).

The following validated index were derived: HOMA-IR [24], insulin sensitivity index according to Matsuda [25], insulinogenic index [26], and disposition index [27].

Lipid analysis included total-cholesterol (t-CHO), high density lipoprotein-cholesterol (HDL-CHO), low density lipoprotein-cholesterol (LDL-CHO), and triglyceride (TG) levels. Glucose plasma concentration, t-CHO, HDL-CHO, LDL-CHO, and TG were measured by an enzymatic method (Roche Molecular Biochemicals, Mannheim, Germany). Insulin and C-reactive protein plasma levels were measured by a Cobas Integra 800 Autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA).

Leptin plasma concentrations were determined by biotinylated polyclonal Gas6 enzyme-linked immunosorbent assay (ELISA, using a commercial kit, according to the manufacturer's instructions (Mediagnost, Reutlingen, Germany).

Body composition was evaluated according to percentage of fat and fat-free mass. Fat mass, fat-free mass, total body water, and extracellular water were determined by bioelectrical impedance analysis (BIA 101/S; Akern, Florence, Italy). Analysis was performed using Bodygram software version 1.2 (Akern). This evaluation was not possible in case of weight >125 kg; over this threshold, the analysis was performed by a dual-energy X-ray absorptiometry (DEXA; GE-Lunar, Madison, WI, USA).

The resting energy expenditure kcal/24 h was determined at 22 to 24°C by indirect calorimetry, as previously described [28], evaluating oxygen and carbon dioxide expenditure by ventilatory canopy (Sensormedics, Milano) every minute for 30 min. The result was referred to 24 h, and the predicted resting energy expenditure was calculated according to the Harris-Benedict formula [29].

Plasma Measurement of Gas6 Protein

Our research used an immunoassay (ELISA) specifically designed by us in our laboratory to quantify the Gas6 protein in human plasma and adhered to the guidelines established by the Food and Drug Administration (FDA) for the validation of the bioanalytic method.

Reagents and Equipment

The primary reagents used in our dosage included a goat polyclonal anti-Gas6 capture antibody and a biotinylated polyclonal Gas6 detection antibody, both procured by research and development systems, Minneapolis, USA. The development system utilized a streptavidin-peroxidase conjugate and 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate, both sourced from Sigma-Aldrich, St. Louis, MO, USA.

To ensure precision and consistency, we used the immunoassay plate Nunc Maxisorp F96 for the preparation of the plate and a spectra count (BS1000 Packard Spectra Count, Meriden, CT, USA) for the readings of optical density at 450 Nm, with a wavelength length reference set at 570 Nm.

Methodology

Our ELISA method provided for a night incubation of the ELISA plates with the capture antibody, followed by blockers and subsequent incubations with plasma or standard samples, detection antibody, and conjugated of streptavidin-peroxidase. The enzymatic reaction was displayed using the TMB substrate, and the reaction was finished with sulfuric acid. The absorbance was therefore measured, providing quantitative results of the Gas6 concentration in plasma samples.

Validation Studies

The validation of our ELISA method was completed, including tests for sensitivity, specificity, precision, accuracy, and reproducibility. We carried out assessments of inter- and intra-test variability, demonstrating variation coefficients constantly below 15%, aligning with the FDA standards. Recovery experiments were conducted to verify the accuracy of our method, with recoveries close to 100% of the values provided when known amounts of Gas6 were spiked into plasma samples.

The lower limit of quantification for our method has been rigorously established to ensure that Gas6 concentrations above this limit are detected with consistent precision and reproducibility.

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This lower limit of quantification was appropriate to detect physiological concentrations of Gas6 in human plasma, making our test suitable for both clinical research and diagnostic applications [30]. This method has already been used in several clinical settings [31,32].

Gas6 Expression in Adipose Tissue

We also collected samples of adipose tissue; more specifically, obese patients underwent a needle biopsy of subcutaneous adipose tissue, as previously described [33]. Conversely, a sample of visceral and subcutaneous adipose tissue was collected in controls during abdominal surgery. These samples have been used to evaluate Gas6 expression at a local level.

Briefly, total mRNA was extracted using a TRIZOL Plus RNA Purification kit (Ambion by Life Technologies, Carlsbad, CA, USA). A superscript VILO cDNASynthesis kit (Invitrogen by Life Technologies, Carlsbad, CA, USA) was used to retrotranscribe 100 ng of mRNA, and cDNA was stored at -30°C. A gene expression assay was performed by real time qPCR using specific Taqman probes (Life Technologies; GAS6: Hs00181323_m1; housekeeping glucuronidase beta: Hs9999908_m1). GAS6 mRNA expression relative to control samples (subcutaneous adipose tissue from healthy controls) were determined using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

All anthropometric, clinical, and biochemical data were systematically entered into a database and analyzed using MedCalc software version 18.10.2 (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium).

Initially, the distribution of each continuous variable was assessed using the Shapiro-Wilk test. This test is particularly sensitive to deviations from a normal distribution in small to medium sample sizes, making it suitable for our study's dataset. The results indicated a non-normal distribution for most of the investigated variables.

Given the non-normality of the data, measures of central tendency and variability were expressed as medians and interquartile ranges, respectively. These measures provide a more accurate reflection of the center location and spread of data in non-normally distributed datasets, as they are less affected by outliers and skewed data than means and standard deviations.

For the comparative analysis between the case and control groups, we used the Mann-Whitney U test. This nonparametric test was chosen because it does not assume a normal distribution of the data and is ideal for comparing differences in medians between 2 independent groups.

Correlation analyzes were conducted using the Spearman rank correlation coefficient to assess the strength and direction of association between continuous variables. This test is used instead of Pearson correlation because it does not assume a linear relationship or normally distributed data, thus providing a more reliable correlation measure for nonparametric data.

Furthermore, we used a general linear model to investigate the effects of multiple covariates on the relationship between Gas6 levels and various anthropometric measures. The general linear model approach was selected due to its flexibility in handling different types of dependent variables and its ability to control for multiple independent variables simultaneously, thus allowing us to adjust for potential confounders in our analysis.

The significance level for all statistical tests was set at a 2-sided *P* value of 0.05. This threshold was chosen to minimize the likelihood of type I errors while still allowing reasonable sensitivity to detect true associations within the data.

Results

Characteristics of the Population

The main features of the study population are reported in **Table 1**. As expected, patients with obesity are characterized by a significantly higher BMI and leptin than lean controls; likewise, fasting plasma glucose, fasting plasma insulin, and HOMA-IR were significantly higher in patients with obesity.

Plasma Concentrations of Gas6

When we looked at Gas6 plasma levels, we observed higher concentrations in patients with obesity (**Table 1, Figure 1**). Gas6 was not influenced by sex (25.5 [21.90-29.50] in females vs in 25.4 [21.7-29.2] in males; *P*=0.94), while conversely it was inversely related to age (*P*=-0.290; *P*=0.016).

In the whole population, Gas6 increased for increasing body weight (*P*=0.444; *P*=0.0002) and BMI (*P*=0.441; *P*=0.0002); moreover, it was directly related to leptin plasma concentrations (*P*=0.434; *P*=0.0002). In merged sex-stratified data analysis, these associations were only confirmed in females (**Table 2**). No associations were found with waist-to-hip circumference ratio (*P*=0.093; *P*=0.57) or percentage of fat mass (*P*=-0.069; *P*=0.67).

Gas6 was still associated to BMI (rho=0.408, *P*=0.001) and leptin (rho=0.367, *P*=0.002) in a general linear model including age and sex as covariates; conversely, when BMI was added to the model, the association with leptin was lost, suggesting that Gas6 is, possibly, a surrogate parameter of adiposity.

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Table 1. General features of the study population.

	Patients with obesity N=42	Controls N=32	p
Age (years)	38.0 [25.5-47.5]	58.0 [46.0-69.0]	0.0001
Gender (M/F)	17/25	18/14	ns
BMI (kg/m^2)	43.8 [41.5-55.7]	25.3 [23.0-26.06]	**0.0001**
AST (U/L)	19.5 [15.0-23.0]	19.0 [16.0-23.0]	0.94
ALT (U/L)	23.5 [18.0-33.5]	21.0 [16.0-27.0]	0.25
GGT (U/L)	20.0 [16.0-31.5]	26.5 [17.5-45.5]	0.16
t-CHO (mg/dL)	171.0 [148.5-200.0]	187.5 [170.5-209.0]	0.19
HDL-CHO (mg/dL)	47.0 [38.0-53.5]	42.0 [34.0-56.5]	0.63
LDL-CHO (mg/dL)	110.5 [92.5-138.0]	117.0 [96.5-140.3]	0.64
TG (mg/dL)	104.0 [84.0-141.0]	91.0 [69.5-135.5]	0.26
Gas 6 (ng/mL)	27.2 [23.8-31.1]	22.3 [17.5-27.5]	**0.0006**
Leptin (ng/mL)	48.3 [39.1-64.4]	6.1 [2.8-12.0]	**0.0001**
Adiponectin (µg/mL)	7.3 [5.6-10.2]	8.3 [5.9-11.1]	0.35
FPG (mg/dL)	101.5 [90.5-107.5]	88.0 [83.5-95.0]	**0.0012**
FPI (mU/L)	16.2 [11.5-23.4]	6.5 [3.2-10.7]	**0.0001**
HOMA-IR	3.8 [2.3-5.5]	1.93 [0.81-2.83]	**0.0001**

The table reports the general features of the study population. In the left column the values are reported (with the interquartile ranges in square brackets) referring to patients with obesity, in the central column those referring to normal weight controls, in the right column the *P* values (statistically significant values are shown in bold). Gas6 – growth arrest-specific 6; BMI – body mass index; AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – gamma glutamyl-transferase; t-CHO – total cholesterol; HDL-CHO – high density lipoprotein; LDL-CHO – low density lipoprotein; TG – triglycerides; FPG – fasting plasma glucose; 2h-PG – plasma glucose 2 h after glucose load; FPI – fasting plasma insulin; 2h-PI – plasma insulin 2 h after glucose load; HOMA-IR – homeostatic model assessment for insulin resistance; HbA1c – glycated haemoglobin.

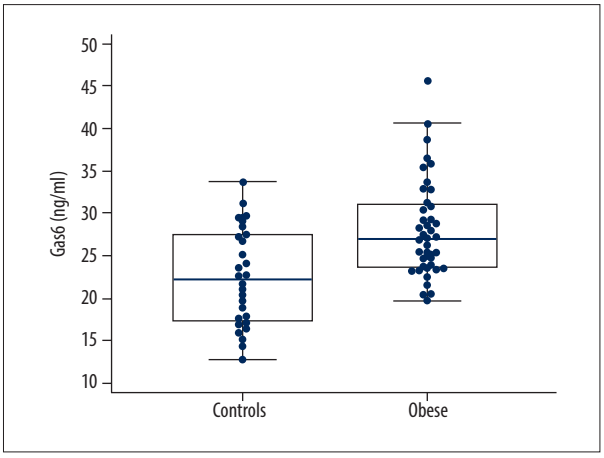

Figure 1. Comparison of plasma growth arrest-specific 6 (Gas6) levels between patients with morbid obesity and those with normal weight. The figure illustrates plasma Gas6 concentration values in patients with obesity (depicted on the right) compared with controls (left) along the x-axis. Gas6 values, expressed in ng/mL, are represented on the y-axis. Box plots represent the median (central horizontal line), interquartile ranges (upper and lower limits of the box), and extreme values (horizontal lines that extend beyond the box). Gas6 – growth arrest-specific 6. *The figure was originally produced by the Authors using the software MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium.*

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Table 2. Correlation values between body mass index (BMI), body weight, and leptin and growth arrest-specific 6 (Gas6) stratified by sex.

Variable	Females N=39	Males N=35
BMI	**(ρ=0.517; p=0.0001)**	(ρ=0.268; p=0.14)
Weight	**(ρ=0.575; p=0.0002)**	(ρ=0.247; p=0.17)
Leptin	**(ρ=0.490; p=0.0002)**	(ρ=0.290; p=0.11)

The table presents correlation values between Gas6 levels and BMI, weight, and leptin concentration in the population, stratified by sex. The left column corresponds to female patients, while the right column corresponds to male patients. Statistical significance of the correlations is observed solely in female patients (P<0.05). Statistically significant values are shown in bold. BMI – body mass index; Gas6 – growth arrest-specific 6.

Gas6 Expression in Adipose Tissue

We then evaluated the levels of Gas6 expression in adipose tissue of patients with obesity and healthy controls. Interestingly, Gas6 mRNA expression was significantly higher in visceral adipose tissue than in subcutaneous adipose tissue in healthy controls; moreover, the subcutaneous expression of Gas6 was similar between patients with obesity and controls (**Figure 2**).

We further evaluated how Gas6 plasma concentration correlated to different metabolic complications of obesity. We first analyzed the associations with glucose metabolism. As shown in **Table 3**, Gas6 was directly associated to HbA1c in the general population; conversely, Gas6 plasma concentration was indirectly related to the insulinogenic index, with an almost significant trend of association with the disposition index. All these associations were strengthened in female sex and completely blunted in male sex.

In contrast, we did not identify any association of Gas6 plasma concentration with lipid metabolism (t-CHO: P=-0.137, P=0.40; HDL-CHO P=-0.038, P=0.82; LDL-CHO P=-0.066, P=0.69; TG: P=-0.214; P=0.19).

Discussion

In the present paper, we investigated the association between severe obesity and Gas6.

The results of our study clarify the complex role of Gas6 in obesity and its associated metabolic disorders, demonstrating significantly high plasma gas levels in individuals with morbid obesity. These results are in accordance with the studies

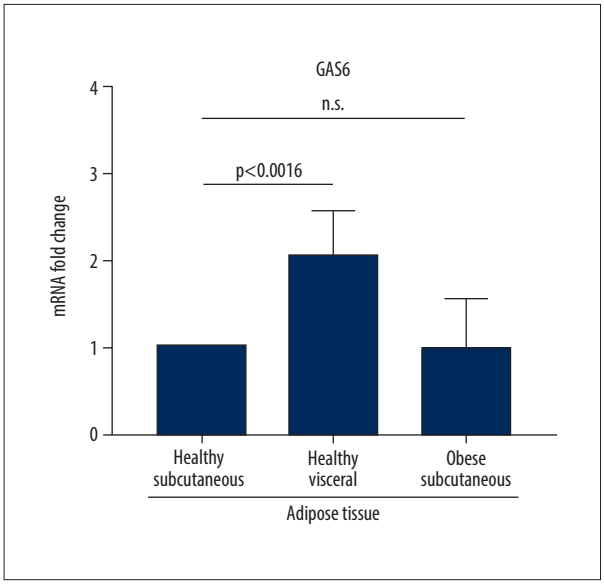

Figure 2. Comparison of growth arrest-specific 6 (Gas6) expression in visceral and subcutaneous adipose tissue of patients with normal weight patients and in patients with morbid obesity. The figure illustrates the mRNA expression of Gas6 (y-axis) across various adipose tissue samples. The subcutaneous adipose tissue of healthy controls is shown to the left of the x-axis, while the middle column displays visceral tissue from healthy controls. The right column depicts subcutaneous tissue from patients with obesity. Significant differences are indicated by a horizontal line above the left and middle columns, denoting a significant distinction. No significant difference was observed between the left and right columns. Gas6 – growth arrest-specific 6. *The figure was originally produced by the authors, using MedCalc Software (Broekstraat 52, 9030 Mariakerke, Belgium).*

conducted by Su et al and Wu et al, which have reported high gas levels of Gas6 in the populations characterized by significant adiposity and insulin resistance [14,16]. This evidence supports the hypothesis that Gas6 can play a regulatory role in the expression of adiponectin and insulin resistance within the adipose tissues, potentially contributing to the metabolic complications often associated with obesity.

In addition, our observations on the higher Gas6 expression in the visceral adipose tissue, compared with subcutaneous deposits, corroborate the results of the study conducted by Su et al [14], highlighting the disproportionate increase of Gas6 in visceral fat as a crucial factor in metabolic dysfunction. The differential expression of Gas6 through various types of adipose tissue clarifies the pathophysiological mechanisms that connect severe obesity to a high metabolic risk.

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Table 3. Correlation between growth arrest-specific 6 (Gas6) levels and glucose metabolism indices stratified by sex.

Variable	General population N=42	Females N=25	Males N=17
HbA1c	**(ρ=0.331; p=0.03)**	**(ρ=0.509; p=0.01)**	(ρ=0.024; p=0.93)
FPG	(ρ=0.021; p=0.90)	(ρ=-0.003; p=0.99)	(ρ=0.108; p=0.69)
2hPG	(ρ=0.192; p=0.24)	(ρ=0.315; p=0.13)	(ρ=-0.056; p=0.84)
FPI	(ρ=-0.052; p=0.75)	(ρ=-0.146; p=0.50)	(ρ=-0.029; p=0.91)
2hPI	(ρ=-0.260; p=0.11)	(ρ=-0.176; p=0.41)	(ρ=-0.321; p=0.23)
HOMA-IR	(ρ=-0.037; p=0.82)	(ρ=-0.123; p=0.57)	(ρ=0.029; p=0.91)
DI	(ρ=-0.301; p=0.07)	**(ρ=-0.449; p=0.03)**	(ρ=-0.187; p=0.52)
ISI	(ρ=0.073; p=0.67)	(ρ=0.067; p=0.76)	(ρ=0.121; p=0.68)
IGI	**(ρ=-0.363; p=0.03)**	**(ρ=-0.458; p=0.03)**	(ρ=-0.262; p=0.37)

The table illustrates the association between Gas6 levels and indices/parameters of glucose metabolism. In the left column, data relating to female patients are presented, while in the right column, data relating to male patients are presented. Gas6 – growth arrest-specific 6; HbA1c – glycosylated haemoglobin; FPG – fasting plasma glucose; 2hPG – 2-h plasma glucose; FPI – fasting plasma insulin; 2hPI – 2-h plasma insulin; HOMA-IR – homeostatic model assessment for resistance to insulin; DI – disposition index; ISI – insulin sensitivity index; IGI – insulinogenic index.

Furthermore, in line with previous studies [16], our research underlines the potential of Gas6 as a biomarker for the inflammation associated with the visceral phenotype of obesity, indicating its usefulness for the diagnostic or prognostic evaluation and as a potential therapeutic objective in the management of the obesity.

It is important to emphasize that high levels of Gas6 were strongly related to the BMI and the plasma concentrations of leptin, suggesting that Gas6 can play a significant role in the metabolic discomfort observed in obesity. In addition, our study introduces further food for thought, considering the impact of sex and age on Gas6 levels. We found a coherent relationship through these demographic data but these correlations have been particularly pronounced among the participating women, highlighting potential specific mechanisms of sex in the role of Gas6 in obesity.

From its discovery in the early 90s, the involvement of Gas6 in human physiology and disease has been widely investigated. This protein seems to be particularly relevant in the regulation of inflammation, fibrosis, and cell proliferation; indeed, targeting the Gas6/TAM axis has been postulated as a new therapeutic strategy in different human conditions, such as cancer, autoimmune diseases, liver cirrhosis, and pulmonary fibrosis [34-39].

We are still on our way to the understanding of this pleiotropic biological system; nonetheless, a growing amount of evidence is now available on the association between Gas6 and obesity. As previously said, in our study, Gas6 plasma levels were increased in patients with obesity when compared with lean controls, being directly correlated to BMI. This observation relates to the recent finding of Holden et al [40], who reported that Gas6 concentrations are higher in obese patients than in lean patients, in a cohort of patients referred for cardiac angiography. Similarly, in a previous cohort of 832 adolescents [15], Gas6 progressively increased from lean to overweight and finally to obese patients. Consistently, Gas6 was also related to BMI. We have to acknowledge that our controls were significantly older than patients with obesity, and considering that Gas6 is inversely related to age, it could be argued that this may have biased our findings. However, in a multivariate analysis, we have demonstrated that Gas6 is independently related to BMI and not to age. Therefore, Gas6 seems to actually behave as a marker of adiposity. Indeed, as previously reported by Stepan et al in 2013, Gas6 is also directly associated to leptin plasma concentrations [41]. However, when BMI is considered as a covariate in a general linear model, this association is lost, suggesting that obesity is the real linkage between these 2 relevant molecules.

We also aimed to understand why Gas6 levels are increased in obesity. Is Gas6 directly produced by fat tissue? Or, on the contrary, is it produced elsewhere, potentially contributing to obesity as a pathogenetic factor?

To try to answer to these questions, we evaluated the expression of Gas6 by rt-PCR in fat tissue; although we did not have the opportunity to collect samples of visceral tissue from patients with obesity, some interesting inferences could be

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indirectly gathered from our data. First, Gas6 was expressed by fat tissue, which is therefore a plausible source of this protein, as supported by preliminary data on pigs [42]. Moreover, the subcutaneous expression does not differ from obese and lean patients, speculatively suggesting that the overproduction of Gas6 can originate from visceral adipose tissue while it cannot be ruled out that macrophages and other inflammatory cells within the visceral adipose tissue stroma can actively contribute to Gas6 production. As a clue, in lean patients, the visceral expression is significantly higher than subcutaneous expression. It is well known that men and women are characterized by a different fat distribution. For the same BMI, women typically present with approximately 10% higher body fat than men, with a preferential subcutaneous distribution [43]. In women with morbid obesity, there is a significant increase of the visceral amount of fat. This might explain why in the female sex the association between BMI and Gas6 is stronger than in males, probably reflecting the contribution of visceral adipose tissue production.

The current literature also supports the idea that Gas6 might, somehow, contribute to the development of obesity. Patients carrying the GG genotype of *GAS6* (rs8191974) are more prone to develop obesity [44]. It is well known that Gas6 regulates cells proliferation, and recent data suggest a potential activity on adipose tissue, favoring adipogenesis. Indeed, Gas6-deficient mice are protected from fat accumulation when fed with a high-fat diet [9]. Consistently, the targeting of the Axl receptor by the compound R428 inhibits preadipocyte differentiation into mature adipocytes. In a murine model, the oral administration of R428 for 5 weeks to mice kept on a high-fat diet significantly reduces weight gain and subcutaneous and gonadal fat mass [12]. This allows us to hypothesize a potential role for Axl inhibition as a therapeutic strategy for the management of obesity.

However, Gas6 might be implicated also in the development of some of the complications typically paralleling obesity. While failing to show associations between Gas6 and lipid profile, we identified a correlation between this protein and glucose metabolism. More specifically, Gas6 was directly associated to HbA1c, with a more pronounced correlation in female sex. This hypothesis is supported by the evidence that Gas6 gene polymorphisms have been associated with the development of diabetes in the Chinese population [45]. However, the literature on this topic is highly contradictory, with some studies reporting an inverse correlation between Gas6 protein levels with plasma glucose, HbA1c, IR, and inflammatory cytokines among patients with type 2 diabetes and those with obesity, and others reporting a positive correlation between Gas6 protein levels or gene polymorphism with IR and inflammation among obese patients [46]. The reason for this discrepancies is still unclear but affects the possibility of drawing definite conclusions on this topic. Similarly, although sex is potentially implied in modulating the relationship of Gas6/glucose metabolism, how this variable acts is yet to be defined. In fact, in 2014, Kuo et al reported that Gas6 levels were negatively correlated with waist and HOMA-IR, being positively correlated with insulin sensitivity in women, but not in men [47].

In an adipocyte cell model, a direct correlation was observed between Gas6 levels and the expression of adiponectin, a protein known for its association with increased insulin sensitivity after metformin treatment. These findings suggest that Gas6 can serve as a regulator in modulating adiponectin expression, indicating a potential role in establishing an association with insulin resistance within adipose tissues [14].

This study, while providing valuable insights into the role of Gas6 in severe obesity, presents several limitations that warrant consideration. First, the sample size of this study is relatively small, which could limit the generalizability of the findings. However, it is important to note that the study population was a homogeneous group of adults with severe pathological obesity, a relatively rare and specific patient demographic. This specificity adds a depth of detail to the findings that might not be achievable in a more heterogeneously composed sample. Moreover, aligning the sample size with that of the control group of normoweight individuals undergoing non-neoplastic, non-inflammatory abdominal surgery was challenging due to the difficulties in recruiting suitable control subjects.

Another limitation is the age difference between the patients with obesity and the control group. This age bias, inherent in our study design, arose because the patients from the San Giuseppe Hospital of the IRCCS Istituto Auxologico Italiano are typically younger adults undergoing residential rehabilitation for severe obesity. Conversely, the control group recruited from the Maggiore della Carità Hospital in Novara consisted of older individuals, reflecting the typical demographic for abdominal surgery for non-obesity-related conditions. Despite this, we believe that this does not substantially skew the interpretation of our results. The direct correlation of circulating Gas6 levels with BMI, and their association with visceral fat in normoweight subjects, suggests that the impact of age on the study outcomes is minimal, reinforcing the validity of our findings in demonstrating the relationship between Gas6 and obesity markers.

Finally, the absence of visceral adipose tissue samples from the obese cohort is a notable limitation. Collecting these samples would have required invasive procedures, which were not justifiable without clinical indications. Therefore, our understanding of Gas6 expression in visceral fat specifically within the obese population remains incomplete. This gap highlights the need for further studies that could safely include such measures, potentially offering more comprehensive insights into the adipose tissue-specific activity of Gas6.

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In conclusion, Gas6 plasma levels are increased in morbid obesity, probably as a consequence of visceral fat production. This is particularly true in female patients and possibly associated with an alteration of glucose tolerance, although how this system and glucose metabolism interact is yet to be elucidated.

Conclusions

Gas6 plasma levels are elevated in morbid obesity, likely originating from visceral fat production, particularly pronounced in women. This may be associated with altered glucose tolerance, although the intricate interactions between Gas6 and glucose metabolism require further elucidation. Our findings contribute valuable insights into the multifaceted role of Gas6 in obesity and associated metabolic complications, opening avenues for future research and potential therapeutic interventions.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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