Evaluation of cytokine GDF15 as a biomarker of obesity

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ABSTRACT
The high calorie intake resulting from the increased consumption of processed foods, associated with the increase in physical inactivity, is a reflection of the economic and social development that shapes the lifestyle of humans today. Obesity and overweight are consequences of this lifestyle that have become a global public health problem and currently affect about 1.9 billion people worldwide.

1 INTRODUCTION

The high calorie intake resulting from the increased consumption of processed foods, associated with the increase in physical inactivity, is a reflection of the economic and social development that shapes the lifestyle of humans today. Obesity and overweight are consequences of this lifestyle that have become a global public health problem and currently affect about 1.9 billion people worldwide[1]. Obesity is associated with a predisposition to a range of other diseases, including 44% of cases of type 2 diabetes, 23% of ischemic heart diseases and hypertension, and 7 to 41% of certain types of cancer, as well as metabolic and inflammatory disorders[23]. Obesity is characterized by an accumulation of adipose tissue in the body as a result of an energy imbalance associated or not with genetic or endocrine-metabolic disorders that favors the accumulation of triglycerides in fat cells.

Factors produced by subcutaneous tissue, pancreas and gastrointestinal tract act on the central nervous system (CNS) and stimulate the endocannabinoid system, hypothalamus (fatty acid sensors) and cortex, eliciting a neurohormonal response that controls the energy balance in the body[4–6]. An imbalance in this control mediated by peripheral stimuli (subcutaneous tissue, pancreas and gastrointestinal tract) to the CNS will result in an inadequate neurohormonal response, adjusting the equilibrium to a higher weight[7]. Particularly interesting among the mechanisms controlling the energy balance, are factors derived from the subcutaneous tissue. In 1953, Gordon Kennedy postulated that factors produced by the subcutaneous tissue control food intake through hypothalamic circuits[8]. Hervey et al. in 1959[9] and Coleman in 1970[10] demonstrated a similar factor. In 1994, this factor was identified as leptin[11].

GDF15 role as an anti-obesity agent has emerged in 2007 in a study published by Kronenberg et al[12] when its function in adapting the energy intake to metabolic imbalances under stress or disease conditions was first described. GDF15 is a stress responsive cytokine of the TGF-β superfamily that is involved in the regulation of inflammatory and apoptotic pathways in injured tissues and at the onset of diseases[13]. This factor is expressed in a multitude of cells including cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells under normal and pathological conditions. Its levels increase in response to tissue injury[14–16]. GDF15 is synthesized as a pro-peptide of approximately 40 kDa. The amino-terminal portion is cleaved and released as a disulfide-linked active dimeric protein of approximately 30 kDa. The protein is present in the serum of all healthy individuals at an average concentration of about 0.6 ng/ml[16]

Studies have shown that GDF15 reduces food intake in rodents and body weight in patients by the suppression of appetite[17–19]. In GDF15 knock-out model, administration of its recombinant form reduced body weight and improved insulin sensitivity. These findings were attributed to an increase of oxidative metabolism and lipid mobilization in the liver, muscle and adipose tissue[7,20,21].

Elevated serum levels of GDF15 induce anorexia in animals with cancer. This effect is due to a direct action of this circulating cytokine on feeding centers in the brain, which can be reversed by the neutralization of GDF15 mediated by the tumor[17]. In patients with lung cancer, elevated levels of circulating GDF15 were associated with weight loss[22], suggesting that this cytokine may be a key weight and appetite regulator and a potential target for the treatment of cancer-induced cachexia[14]. Recently, GDNF family receptor alpha like (GFRAL) localized exclusively in the rhombencephalon (hind-brain) was identified as a receptor that mediates the effects of GDF15[23–25].

Hypothetically, the levels of GDF15 inversely correlates the obesity degree – with lower GDF15 levels observed in patients with higher obesity degrees. However, the levels of this cytokine are known to increase in response to inflammatory processes. Considering that obese individuals present a constant and systemic inflammatory response, the concentration of GDF15 might be higher

than expected due to a greater number of comorbidities[11,19]. In the case of weight loss diets, a decline in GDF15 would increase appetite and the body would have severe difficulties in losing weight, an effect similar to the well-known leptin mechanism[26].

This study evaluated the use of GDF15 as a parameter for monitoring the obesity status of individuals, questioning its usefulness as a biomarker of successful weight loss treatment.

2 METHODOLOGY

2.1 STUDY POPULATION AND CLASSIFICATION OF OBESITY

Serum samples were obtained from 154 volunteers with different degrees of obesity who attended the Eletro Bonini Hospital (HEB), University of Ribeirão Preto (UNAERP), and the obesity outpatient clinic of the University Hospital of the Ribeirão Preto Medical School, University of São Paulo (HCRP-USP), from June 2017 to March 2019. The study was approved by the UNAERP Research Ethics Committee (Approval No. 2.382.773) and written informed consent was obtained from all participants.

The study inclusion criteria were the patient's body mass index (BMI). The participants were thus divided into five experimental groups as follows: control group, BMI < 29.9 kg/m²; Obesity class I, BMI 30 to 34.9 kg/m²; Obesity class II, BMI 35 to 39.9 kg/m². Obese patients with a BMI higher than 40 kg/m² were analyzed in two groups according to the severity of obesity: Obesity class III, BMI 40 to 44.9 kg/m² and severely obese, BMI > 45 kg/m². Patients who underwent bariatric surgery were excluded from the study.

The presence of comorbidities was also analyzed, and the participants were divided into subgroups without comorbidities and with arterial hypertension, diabetes mellitus and metabolic syndrome.

The GDF15 levels were associated with patient's anthropometric data, including weight, height, neck circumference, waist circumference, hip circumference, waist-to-hip ratio, and body fat percentage (the body fat percentage was calculated according to the method described by Lerario et al.[27]. All participants were also classified based on age, gender, and race.

2.2 MEASUREMENT OF SERUM GDF15 LEVELS

Venous blood samples were collected after a 12-hour fast into EDTA tubes following standard protocols. Serum was separated from whole blood by centrifugation for 10 min at 1,500 rpm and stored at -80°C for subsequent analysis. The levels of GDF15 were quantified in serum or plasma samples using the Human GDF15 ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA) following manufacturer instructions.

2.3 STATISTICAL ANALYSIS

One hundred and fifty-four (154) individuals were recruited for the study. The patients were enrolled at the Electro Bonini Hospital (HEB) and at the University Hospital of the Ribeirão Preto Medical School, University of São Paulo (HCRP-USP). Table I shows the patient distribution according to gender and the degree of obesity.

Table I. Distribution of the participants according to the degree of obesity and gender.

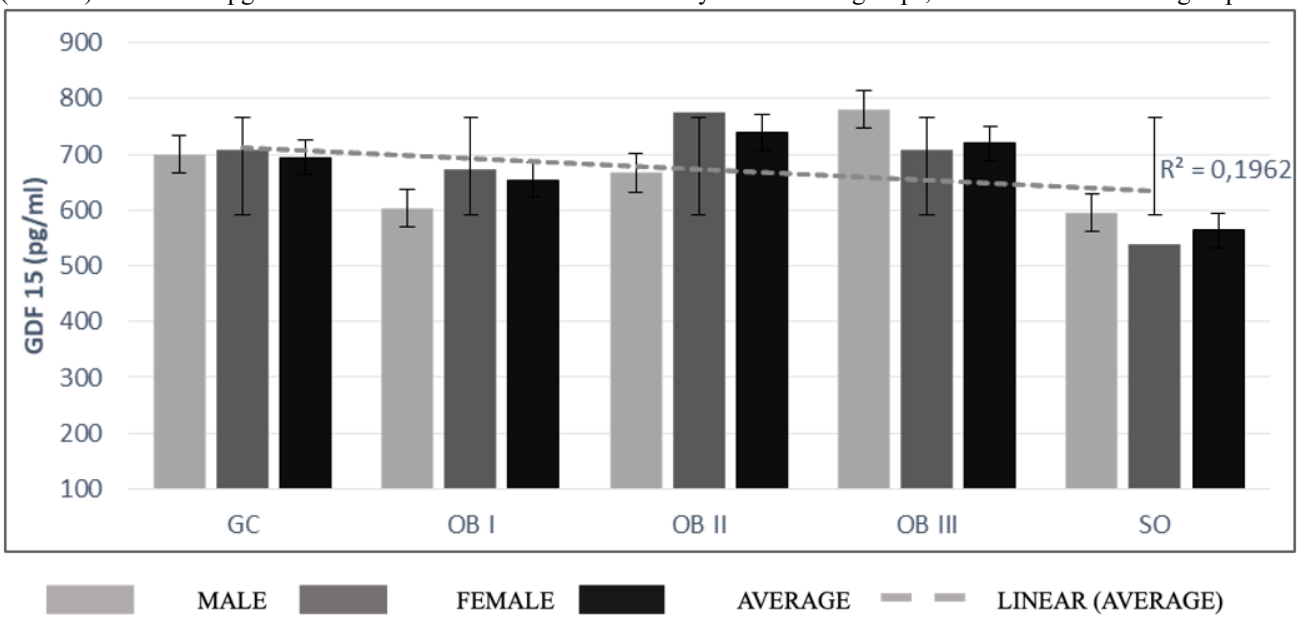
	Male	Female	Total
Control	15 (9.7%)	22 (14.4%)	37 (24%)
Obesity I	7 (4.5%)	20 (12.9%)	27 (17.5%)
Obesity II	8 (5.1%)	17 (11.2%)	25 (16.3%)
Obesity III	5 (3.2%)	23 (14.9%)	28 (18.2%)
Superobese	17 (11.2%)	20 (12.9%)	37 (24%)
Total	52 (33.7%)	102 (66.3%)	154 (100%)

Table II displays the presence of comorbidities in the study participants, in which the degree of obesity (BMI) is correlated with the presence or absence of comorbidities (arterial hypertension, diabetes mellitus, and metabolic syndrome). The parameters analyzed display normal distribution (p>0.05).

Table II. Distribution of the participants according the degree of obesity and presence or absence of comorbidities.

	No comorbidity	AH	DM	MetS	Total	With comorbidities
Control	25	4	7	1	37	32.4%
Obesity I	17	4	2	4	27	37%
Obesity II	15	2	3	5	25	40%
Obesity III	6	12	5	5	28	78.5%
Superobese	0	17	9	11	37	100%

Figure 1. Plasma GDF15 levels (pg/ml) according to the degree of obesity. The mean level in individuals with a BMI < 30 (control) was 694.44 pg/ml. Comorbidities were observed not only in the obese groups, but also in the control group.



In our study, GDF15 levels in obese individuals were exacerbated compared to the control group (p>0.05) (Figure 1). This finding might be due to the increased inflammatory state of these individuals aggravated by the comorbidities observed. The higher GDF15 levels are dissociated from BMI.

It is well stablished that the obese severity directly correlates with an increase in the comorbities observed, some of them related with a GDF elevation, a fact that impairs the demonstration of an inverse correlation. This explains why GDF15 shows an expected, although not expressive, correlation with the degree of obesity (R^2=0.1962).

GDF15 levels would be elevated because of the inflammatory response triggered by these comorbidities or even by severe obesity, not acting effectively on its GFRAL receptors[28].

This fact was clearly observed in the participants when GDF15 levels were compared between individuals of the same group with and without the described comorbidities. GDF15 was expressed above average levels in the presence of arterial hypertension, diabetes mellitus or metabolic syndrome compared to the control group (Table III).

Table III. Mean GDF15 levels in the different BMI groups with or without comorbidities.

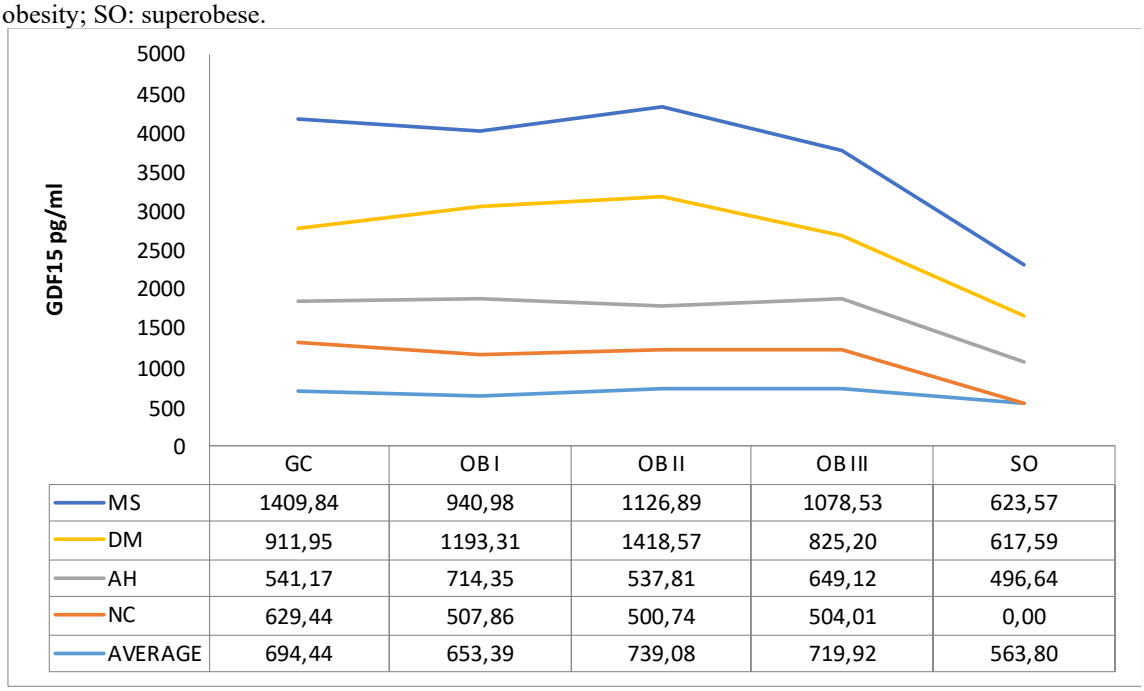
Groups	N (total)	GDF Level	NCOM	GDF NCOM	AH	GDF - AH	DM	GDF - DM	SM	GDF - SM
GC	37	694.44	25	629.44	4	541.17	7	911.95	1	1409.84
MGC	15	699.81	11	698.18	1	588.12	2	876.83	1	1409.84
FGC	22	705.78	14	575.43	3	525.52	5	859.70	0	0.00
OB I	27	653.39	17	507.86	4	714.35	2	1193.31	4	940.98
MOBI	7	603.82	4	449.65	1	725.94	0	0.00	2	851.09
FOBI	20	670.74	13	525.77	3	710.49	2	1193.31	2	1030.88
OB II	25	739.08	15	500.74	2	537.81	3	1418.57	5	1126.89
MOB II	8	666.64	6	495.85	1	481.53	1	1876.53	0	0.00
FOB II	17	773.16	9	504.01	1	594.08	2	1189.59	5	1126.89
OB III	28	719.92	6	474.95	12	649.12	5	825.20	5	1078.52
MOB III	5	780.14	0	0.00	4	796.99	0	0.00	1	712.75
FOB III	23	706.83	6	474.95	8	575.18	5	825.20	4	1169.97
SO	37	563.80	0	0.00	17	496.64	9	617.59	11	623.57
MSO	17	594.69	0	0.00	6	553.37	6	610.68	5	625.07
FSO	20	537.54	0	0.00	11	465.70	3	631.42	6	622.32

Data represented in table III shows distinct GDF levels in the control group. This is possibly due to several aspects related or not with the obesity levels such as gender, race, weight, body fat percentage, as already describe in the current literature[13,29,30]. Hence, the results show that GDF15 levels may vary according to the anthropometric data of the individuals and presence of comorbidities.

Analysis of the curves in Figure 2 obtained from Table III data shows that the mean GDF15 levels inversely correlates with the obesity degrees in the different groups analyzed, with lower levels registered in the higher obesity degree groups (R^2 0.196). Interestingly, the mean GDF15 levels in individuals without comorbidities shows a strong inverse correlation (R^2 0.661) with the obesity

degrees, being lower in individuals with higher degree of obesity, as expected. When analyzed together with the comorbidities evaluated, all groups display lower GDF15 levels according to their respective obesity severity (arterial hypertension: R^2 0.0727, diabetes mellitus: R^2 0.2307, and metabolic syndrome: R^2 0.626). Higher GDF15 levels would also be associated with the morbid state and not only to the expression of this cytokine in these individuals.

Figure 2. Analysis of GDF15 levels in the different obese groups according to the presence or absence of comorbidities. Graphical representation of linear regression and coefficient of determination. NC: no comorbidities; AH: arterial hypertension; DM: diabetes mellitus; MetS: metabolic syndrome; CG: control group; OB I, OB II and OB III: class I, II and III obesity; SO: superobese.



	GC	OB I	OB II	OB III	SO
MS	1409,84	940,98	1126,89	1078,53	623,57
DM	911,95	1193,31	1418,57	825,20	617,59
AH	541,17	714,35	537,81	649,12	496,64
NC	629,44	507,86	500,74	504,01	0,00
AVERAGE	694,44	653,39	739,08	719,92	563,80

Metabolic syndrome is a genetic disorder that affects individuals regardless of their obesity degree. This implies that, in this patients, GDF15 cytokine is expressed without the comorbidities bias seen in the other groups. This is perceived in the MS group analysis , where a strong inverse relationship was observed between GDF15 and the different BMI groups (R^2=0.626).

As expected, GDF15 was lower in the superobese group compared to the other groups. All individuals in this group had at least one comorbidity. Considering the severity of this group, superobese individuals probably have a very low GDF15 expression associated with the obesity degree. The measured GDF15 would only reflect the inflammatory response induced by the morbid states, another evidence of the inverse correlation between GDF15 and the degree of obesity[26].

Taken together, the findings indicate that plasma GDF15 levels are related to the BMI in such a way that its low expression would not stimulate hypothalamic satiety receptors (GFRAL), resulting in an increase of the body's energy reserves and consequent weight gain[26,28,31]. In this study, GDF15 showed very weak correlations with weight and body fat percentage. No correlation was observed with the waist-to-hip ratio, demonstrating the lack of a relationship with the type of obesity, visceral or not.

The present results suggest an inverse relationship between serum GDF15 levels and obesity status. The higher the degree of obesity of the individual, the lower the expression of GDF15. These data would be more evident in individuals without comorbidities. Since GDF15 is a cytokine of the inflammatory response, its levels would be increased in the presence of comorbidities.

3 CONCLUSION

The present results indicate that GDF15 can potentially be used as a biomarker in non-morbid obese patients, where its expression could potentially indicate a predisposition to the obesity state maintenance, as a result of an energetic unbalance due to the satiety absence from the food intake. However, in individuals with comorbidities, GDF15 levels must be compared within their own curve and the morbid state needs to be taken into consideration. One possibility is that the measurement of serum GDF15 levels in younger individuals without comorbidities, who have not yet reached a state of obesity, could generate information about their predisposition to obesity. Lower expression of GDF15 would indicate a higher risk of developing obesity, but the opposite does not apply. Prospective studies are necessary to establish GDF15 as a biomarker to prevent obesity in high-risk groups.

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