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Preliminary data on the relationship between circulating levels of Sirtuin 4, anthropometric and metabolic parameters in obese subjects according to growth hormone/insulin-like growth factor-1 status ☆

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ABSTRACT

Background: The main components of GH/insulin-like growth factor (IGF)-1 axis and Sirtuin 4 (Sirt4), highly expressed in liver and skeletal muscle mitochondria, serve as active regulators of mitochondrial oxidative capacity with opposite functions. In obesity both GH/IGF-1 status and serum Sirt4 levels, likely mirroring its reduced mitochondrial expression, might be altered.
Objective: To evaluate the association between circulating levels of Sirt4, body composition, metabolic parameters and cardio-metabolic risk profile in obese patients according to their different GH/IGF-1 status.
Design: Cross-sectional study with measurement of serum Sirt4, GH after GH releasing hormone (GHRH) + Arginine test, IGF-1 and assessment of body composition, glucose and lipid metabolism in 50 class II–III obese subjects (BMI 35.6 to 62.1 kg/m^2) and 15 normal weight subjects. Low GH secretion and IGF-1 were defined using pre-determined cutoff-points. The Homeostatic Metabolic Assessment of insulin resistance index and Visceral adiposity index were also calculated. The association of Sirt4 with peak stimulated GH and IGF-1, body composition, metabolic parameters and cardio-metabolic risk profile was assessed.
Results: Serum Sirt4 was inversely related to anthropometric and metabolic parameters and positively related to peak GH and IGF-1. After adjusting for peak GH and IGF-1, the relationships between Sirt4 and BMI became not significant. At multiple regression analysis IGF-1 ($p < 0.001$) was the independent predictor for Sirt4.
Conclusion: There was a close relationship between low IGF-1 and low serum Sirt4. This observation suggested that in obese patients, low GH/IGF-1 status was likely associated with a major compensatory decrease in circulating levels of Sirt4 to oppose to its negative regulator effect on mitochondrial oxidative capacity.

1. Introduction

Apart from their effects on linear growth before puberty, growth hormone (GH) and insulin-like growth factor (IGF)-1, the main components of the GH axis, have defined anabolic functions and act as key regulators of energy metabolism in adulthood [1]. Actually, the interaction between the GH axis and body weight can be viewed as part of the overall regulation of body composition across the feeding/fasting cycle, being adipose tissue and skeletal muscle as major targets of the action of the GH/IGF-1 axis. Of interest, GH and IGF-1 receptors expressed on skeletal muscle have been reported to regulate basal skeletal muscle lipid oxidation [2] and to promote mitochondrial oxidative capacity [3,4]. Albeit mainly reversible after calorie restriction and sustained weight loss [5], the low GH/IGF-1 status in obese individuals might be one of the factors accounting for heterogeneity in metabolic phenotype among equally obese subjects, as this subset of obese subjects presented with higher prevalence of cardio-metabolic risk factors [6] and worse body composition compared with obese individuals with a normal GH/IGF-1 axis [7]. In particular, we previously reported that up to 1/3 of severely obese patients exhibited a low GH/IGF-I status associated with significantly higher fat mass and waist circumference [8] or metabolic syndrome prevalence [9].

☆ *SS* conceived the study and drafted the manuscript. *GT* contributed to drafting the manuscript and carried out the statistical analysis of the data. *CDS*, *LB*, and *CF* performed laboratory analyses. *FO* and *FP* collected the data. *AC* and *FC* critically revised the text.

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Sirtuins are NAD+-dependent enzymes that couple cellular energy production to metabolic demand [10]. Sirtuin 1 and Sirtuin 4 (Sirt4) are involved in insulin secretion [11] and in maintaining the balance of glucose/lipid metabolism in type 2 diabetes mellitus (T2DM) [12]. Sirt4, ubiquitously expressed in many organs including skeletal muscle and the liver, functions within the mitochondria as negative regulator of mitochondrial oxidative capacity [13]. Evidence now indicates that the increased oxidative stress, due to excess visceral obesity, as the main source of cytokines and adipokines [14], is one of the factors involved in mitochondrial dysfunction [15]. Very recently, we evidenced that circulating levels of Sirt4, likely mirroring its reduced mitochondrial expression, are low in obese patients, likely as an attempt to preserve in obesity the mitochondrial oxidative capacity, and are associated with cardiovascular risk factors [16]. Aside from Sirt4, also GH has been reported to actively regulate mitochondrial oxidative capacity. Actually, both low GH/IGF-1 status and low serum Sirt4 levels could occur concomitantly in obesity. However, the possible relationship between Sirt4 and anthropometric and metabolic parameters among obese subjects according to GH/IGF-1 status has not been investigated formerly.

The aim of the present study was to evaluate the association between circulating levels of Sirt4, body composition, metabolic parameters and cardio-metabolic risk profile in obese patients according to their different GH/IFG-1 status.

2. Materials and methods

2.1. Study design and population

In order to calculate the minimum required sample the means of Sirt4 were evaluated. Out of the 97 consecutive morbidly obese subjects referred to the out-patient Endocrinology Unit, from January 2013 to December 2013, 50 obese subjects were enrolled in this cross-sectional study. All subjects underwent complete medical history and clinical examination and the selection was based on GH/IGF-1 status as assessed during their screening procedure for bariatric surgery by evaluating peak GH after the GHRH + Arginine (ARG) stimulation test and IGF-1 circulating levels as indicators of GH sufficiency. Subjects were eligible if they presented with established obesity (at least three years), and were on a balanced low calorie, low fat (25% of calories) diet (lasting at least three months). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a previously calibrated mercury sphygmomanometer, and the mean value of three measurements was calculated. Exclusion criteria were: 1) presence of T2DM [17]; 2) presence of liver or renal failure, cancer, and acute or chronic inflammatory diseases based on a complete medical examination and laboratory investigations; 3) presence of additional pituitary hormone deficiency, specifically: a) diabetes insipidus was ruled out by normal urine volume (<2.5–3 l/24 h) with normal urine osmolality (>300 mmol/kg); b) normal adrenal function was demonstrated by early-morning (at 9.00 am) cortisol concentrations more than 80 μg/l and 24 hour urinary free cortisol levels >30 μg/24 h; c) absence of secondary hypothyroidism was demonstrated by normal FT4 (>8 ng/l) concentrations with normal TSH levels; d) the absence of secondary hypogonadism was demonstrated by: i) in premenopausal women with normal menstrual patterns (i.e. at least 10 menstrual periods in the previous year, the last less than 60 days before the 1st examination), normal estradiol levels (>20 pg/ml) with normal FSH and LH levels; ii) in men by normal testosterone levels (>3 μg/l) with normal FSH and LH levels; 4) subjects on weight loss medications, or hormonal medications including estrogen, testosterone, glucocorticoids, anabolic steroids, GHRH, GH, IGF-1, hormonal contraception, or other drugs (i.e., aspirin, metformin, statins and fibrates) known to possibly alter laboratory data, were excluded. Fifteen lean, apparently healthy, subjects (screened for the entire laboratory panel and instrumental parameters used for the obese individuals) were chosen as controls to avoid any confounding factor with circulating Sirt4 levels. The study was conducted without support from the pharmaceutical industry, after approval by the institutional review board of the University of Naples, Italy (n. 5/14). As control group 15 Caucasian normal weight subjects (5 males, mean age 33.4 ± 7.8 years) were chosen between volunteers and employees of our Institution, with comparable education, income, marital status, smoking habits, or participation in physical activity. The purpose of the protocol was explained to the patients and an informed written consent was obtained from each patient at the beginning of the study.

2.2. Assay methods

Samples were collected in the morning between 8 and 10 a.m., after an overnight fast of at least 8 h and stored at −80 °C until being processed. All biochemical analyses including fasting plasma glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and transaminases were performed with a Roche Modular Analytics System in the Central Biochemistry Laboratory of our Institution. LDL cholesterol and HDL cholesterol were determined by a direct method (homogeneous enzymatic assay for the direct quantitative determination of LDL and HDL cholesterol). Glycated hemoglobin (HbA1C) was measured by HPLC. In premenopausal women hormonal investigations were performed during the early follicular phase, 5–7 days after spontaneous menses. Serum GH levels were measured by immunoradiometric assay (IRMA) using commercially available kits (HGH-CTK-IRMA, Sorin, Saluggia, Italy). The sensitivity of the assay was 0.2 μg/l. The intra- and interassay coefficients of variations (CVs) were 4.5 and 7.9%, respectively. Plasma IGF-1 was measured by IRMA after ethanol extraction. The sensitivity of the assay was 0.8 μg/l. According to our population reference values [18], the normal IGF-1 range in 20–40, 41–60, and over 60-year-old subjects was 110–494, 100–300, and 78–260 μg/l, respectively. The intra-assay CVs were 3.4, 3.0, and 1.5% for low, medium, and high points on the standard curve, respectively. The inter-assay CVs were 8.2, 1.5, and 3.7% for low, medium, and high points on the standard curve, respectively. Total insulin levels were measured by a solid-phase chemiluminescent enzyme immunoassay using a commercially available kit (Immunolite Diagnostic Products Co, Los Angeles, CA). The intra-assay coefficients (CV) of variations were less than 5 · 5% for insulin assay. Insulin resistance was determined by the Homeostasis model assessment of insulin resistance (HoMA-IR), which was assessed by the formula: fasting insulin (μU/ml) × fasting glucose (mg/dl) / 405, according to Matthews et al. [19]. Visceral adiposity index (VAI) score was calculated using the gender-specific reported formula [20] and was (waist circumference / 39.68 + (1.88 × BMI)) ×(triglycerides / 1.03) × (1.31 / HDL cholesterol).

2.3. Assessment of GH secretion

The GHRH + ARG test was performed after an overnight fast according to Ghigo et al. [21]: ARG (arginine hydrochloride, Salf, Bergamo, Italy) was administered at a dose of 0.5 g/kg, up to a maximal dose of 30 g, slowly infused from time 0 to 30 min, while GHRH (1-29, Geref, Serono, Rome, Italy) was given at a dose of 1 μg/kg as i.v. bolus at time 0. Blood samples were taken every 15 min from −15 up to 90 min. A cut-off value of ≤4.2 μg/l was used for peak stimulated GH, based on Corneli et al. [22]. IGF-1 z score or standard deviation score (SDS) for age and gender was also calculated according to our population reference values [18] and used for further analysis. For the purpose of this study, IGF-1 levels were classified as “normal” when higher than −2.0 SD and “deficient” when lower than −2.0 SD [23].

2.4. Determination of circulating Sirt4 levels

The kit for in vitro quantitative measurement of Sirt4 in human tissue homogenates and other biological fluids, based on a sandwich enzyme immunoassay, was provided by Uscn Life Science & Technology

Company, 3603 DOUBLE LAKE DR, Missouri City, TX 77459. The detection range was 0.156–10 ng/ml, as reported elsewhere [16]. Briefly, the standard curve concentrations used for the ELISA were 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml, and 0.156 ng/ml. The sensitivity of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. The minimum detectable dose of human Sirt4 was less than 0.051 ng/ml. No significant cross-reactivity or interference between human Sirt4 and other sirtuins was observed, according to manufacturer data. Intra-assay precision was determined as follows: three samples with low, middle and high level human Sirt4 were tested 20 times on one plate. The inter-assay precision was weighed testing three samples with low, middle and high level human Sirt4 on 3 different plates, 8 replicates in each plate. The coefficient of variation, calculated by SD/mean × 100 for the intra-assay and inter-assay was <10% and <12%, respectively.

2.5. Anthropometric measurements

All anthropometric measurements were taken with subjects wearing only light clothes and without shoes. In each men, weight and height were measured to calculate the BMI [weight (kg) divided by height squared (m^2), kg/m^2]. Height was measured to the nearest cm using a wall-mounted stadiometer. Body weight was determined to the nearest 50 g using a calibrated balance beam scale. The degree of obesity was established on the basis of BMI cut-off points of 30–34.9 (class I obesity), 35–39.9 (class II obesity) and ≥40 kg/m^2 (class III obesity), respectively. Excess Body Weight (EBW) was calculated as actual weight − ideal weight. Visceral obesity was identified by measuring waist circumference using a flexible steel tape measure at the natural indentation or at a level midway between the iliac crest and the lower edge of the rib cage if no natural indentation was visible. Body composition was performed in each patient with bioimpedance analysis (single-frequency 50 kHz BIA 101 RJL, Akern Bioresearch, Firenze).

2.6. Statistical analysis

The minimum required total sample was calculated using the pooled standard deviation with the level test 0.05 and power 90% of Sirt4 in obese patients and controls. Study variables are shown as mean ± standard deviation (SD) or as median plus range according to variables' distribution analyzed by the Kolmogorov–Smirnov test. To correct for skewed distributions, Sirt4 and peak GH values were logarithmically transformed and back-transformed for presentation in tables and figures. Differences in serum Sirt4 levels between obese subjects and normal weight controls were analyzed by Mann–Whitney *U* test. Pearson's correlation coefficients and partial correlation coefficients were used to assess the relationship between serum concentrations of Sirt4, body composition, metabolic parameters, peak GH and IGF-1 levels. To assess the independent effect of a quantitative variable on the prediction of another one, the linear regression analysis (least squares) was used, evaluating the coefficient with its standard error, 95% confidence intervals (CI), the t (t-stat), and p values. A t-stat greater than 1.96 with a significance less than 0.05 indicates that the independent variable is a significant predictor of the dependent variable within and beyond the sample. A multiple linear regression analysis model (stepwise method) was used to estimate the predictive value of peak GH, IGF-1 SDS and waist circumference, expressed as R^2, Beta (β) and t with Sirt4 as dependent variable. In this analysis, we entered only those variables that had a p-value <0.05 in the univariate analysis (partial correlation). To avoid multicollinearity, variables with a variance inflation factor (VIP) >10 were excluded. Data were stored and analyzed using the MedCalc® package (Version 13.0 — © 1993–2014 MedCalc Software bvba — MedCalc Software, Mariakerke, Belgium).

3. Results

When calculating the minimum required sample, evaluating the difference of mean of Sirt4 with a type I error of 0.10 and a type of II (β) error of 0.05, the resulting size of the groups was 48 and 15, respectively. Of the initial 97 participants, eight T2DM patients on treatment with metformin and nine patients on lipid lowering drugs (two on statins, three on fibrates and four on statin–fibrate combination for mixed hyperlipidemia) were excluded. Eleven obese subjects were excluded because they had undergone steroid therapy (five for inflammatory bowel disease, three for Chronic Obstructive Pulmonary Disease, two for severe psoriasis, one for rheumatoid arthritis). Three patients with Obstructive Sleep Apnea Syndrome, seven males with obesity-related hypogonadism, and nine obese women taking contraceptives were also excluded. Characteristics of the 50 obese subjects participating in the study are summarized in Table 1. All presented with class II–III obesity, with BMIs ranging from 35.6 to 62.1 kg/m^2. Eleven patients were

Table 1
Clinical and metabolic characteristics.

Number of patients	50
Demographics	
Gender (F/M)	33/17
Age (years)	37.1 ± 9.7
Body composition	
BMI (kg/m^2)	46.4 ± 6.0
VAI	5.5 ± 3.5
EBW (kg)	70.3 ± 19.8
Waist circumference (cm)	148.6 ± 14.9 (M) 134.3 ± 18.9 (F)
Fat mass (kg)	58.9 ± 14.1 (M) 61.2 ± 18.1 (F)
Fat mass (%)	40.1 ± 5.7 (M) 48.3 ± 6.9 (F)
Free fat mass (kg)	81.6 ± 21.4 (M) 63.3 ± 8.3 (F)
Free fat mass (%)	59.9 ± 5.7 (M) 55.7 ± 6.9 (F)
Metabolic parameters	
Fasting plasma glucose (mg/dl)	97.5 ± 13.7
Fasting insulin (μU/ml)	21.2 ± 9.5
HoMA-IR	5.2 ± 2.6
HbA1C	5.9 ± 0.4
Total cholesterol (mg/dl)	194.6 ± 36.6
HDL cholesterol (mg/dl)	45.9 ± 11.8 (M) 46.1 ± 11.0 (F)
LDL cholesterol (mg/dl)	116.4 ± 31.2
Triglycerides (mg/dl)	149.8 ± 88.1
AST (U/l)	23 (10–109)
ALT (U/l)	35 (9–202)
γGT (U/l)	30 (11–242)
SBP (mm Hg)	131.7 ± 14.1
DBP (mm Hg)	83.5 ± 10.1
GH status	
Peak GH (ng/ml)	2.6 (0.05–10)
Peak GH < 4.2 ng/ml	30 (60%)
IGF-1 (ng/ml)	138.0 ± 38.9
IGF-1 SD < 2.5	29 (58%)
Low Peak GH and IGF-1 SDS	31 (62%)

Values are mean ± SD except for liver function tests, and peak GH which are reported as median (range). Peak GH after GH releasing hormone (GHRH) + Arginine test was performed according to Ghigo et al. [21]. IGF-1 z score or standard deviation score (SDS) for age and gender was also calculated according to our population reference values [18]. Low GH secretion [22] and low IGF-1 levels [23] were defined using pre-determined cut-off-points. HoMA-IR and VAI were also calculated, according to Matthews et al. [19] and Amato et al. [20], respectively.

BMI, Body Mass Index; *VAI*, Visceral adiposity index; *EBW*, Excess Body Weight; *HoMA-IR*, HoMA-IR, Homeostasis model assessment of insulin resistance; *HbA1C*, glycated hemoglobin; *AST*, Alanine Transaminase; *ALT*, Aspartate Transaminase; *γGT*, Gamma-glutamyltransferase; *SBP*, systolic blood pressure; *DPB*, diastolic blood pressure; *GH*, growth hormone; *IGF-1*, insulin-like growth factor-1; *IGF-1 SDS*, IGF-1 z score or standard deviation score.

treated for hypertension and 26 patients had impaired fasting glucose levels greater than 110 mg/dl but less than 126 mg/dl. Serum Sirt4 in obese subjects were significantly lower than in normal weight subjects: 0.38 (0.05–10.16) ng/ml vs 6 (1.18–20.00) ng/ml ($p = 0.001$; Fig. 1), without any significant gender differences ($p = 0.556$).

Assuming 4.2 ng/ml to be the peak GH cut-off point established for the GHRH + ARG test in obese individuals cut-off points for diagnosing GHD and IGF-1 SDS ≤ −2.0 for diagnosing IGF-1 deficiency, more than one half of obese subjects in this series presented with low peak GH or low IGF-1 levels, with a 60% concordance rate of low peak GH and low IGF-1. When obese subjects were grouped according to their GH/IGF-1 status, obese subjects with low peak GH and/or low IGF-1 levels (31 subjects), presented with lower Sirt4 (0.29 (0.05–3.3) vs 2.5 (0.08–10.16)), and higher values of BMI (48.6 ± 6.1 vs 42.9 ± 4.0; $p = 0.001$), waist circumference (145.8 ± 15.8 vs 129.8 ± 18.7; $p = 0.002$), VAI (6.4 ± 4.0 vs 3.9 ± 1.9; $p = 0.011$), and HoMA-IR (6.1 ± 2.4 vs 3.6 ± 2.1; $p < 0.001$) than obese subjects (19 subjects) with normal GH/IGF-1 status, albeit there were no age differences between the two groups (36.2 ± 9.1 vs 38.4 ± 10.7; $p = 0.441$).

3.1. Correlation studies

Table 2 shows the correlations between Sirt4 and the study variables. These data demonstrate the presence of significant associations between serum Sirt4 and BMI, waist circumference, fat mass, EBW, VAI, insulin, triglycerides, total cholesterol, LDL cholesterol, DBP, peak GH, and IGF-1 SDS. Otherwise, no significant relationship was detected between Sirt4 and age ($p = 0.140$), fasting glucose ($p = 0.570$), HoMA-IR ($p = 0.083$), HDL cholesterol ($p = 0.152$), liver function tests (AST: $p = 0.563$; ALT: $p = 0.487$; *γGT*: $p = 0.065$), and SBP ($p = 0.570$). Because of the presence in our series of two conditions known or suspected to affect Sirt4 levels, such as insulin levels and low GH/IGF-1 status, respectively, the correlation coefficients were adjusted for insulin, peak GH or IGF-1 levels. When this was done, in the first case Sirt4 still remained significantly correlated with BMI, the majority of anthropometric variables, peak GH and IGF-1; whereas after adjusting for peak GH and IGF-1, the relationships between Sirt4 and BMI became not significant, and only waist circumference and VAI maintained their significant association with Sirt4 (Table 2). Fig. 2 (a, b, c, d) shows the significant prediction of anthropometric variables, peak GH or IGF-1 levels as independent variables on serum Sirt4. The prediction of lower Sirt4 levels by major severity of BMI, higher waist circumference and lower peak GH and IGF-1 was well documented. Consequently, we performed a multiple regression analysis to determine between anthropometric variables, peak GH or IGF-1 levels the major predictor of Sirt4. The data reported in Table 3 show that when waist circumference, peak GH and IGF-1 were forced into the model together, being BMI, VAI and EBW not present due to their multicollinearity with waist circumference, IGF-1 ($p < 0.001$) was independently related to Sirt4, while waist circumference and peak GH lost their independent predictive power, highlighting the close relationship between low IGF-1 and low serum Sirt4 (for the entire model, $R^2 = 0.407$).

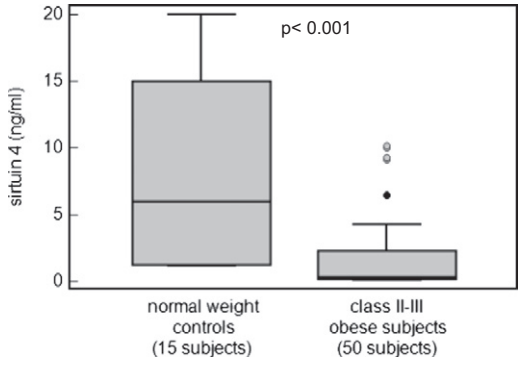

Fig. 1. Behavior of serum circulating Sirtuin 4 (ng/ml) in normal weight controls and the study population of class II–III obese individuals. To correct for skewed distributions, Sirtuin 4 values were logarithmically transformed and back-transformed for presentation in the figure. *Sirtuin 4*, Sirt4.

Table 2
Correlation coefficients between Sirtuin 4, anthropometric and metabolic variables, GH and IGF-1.

	Bivariate correlation	Partial correlation[a]	Partial correlation[b]
BMI	**−0.487**	**−0.368**	−0.263
	<0.001	*0.010*	*0.071*
Waist circumference	**−0.552**	**−0.494**	**−0.354**
	<0.001	*<0.001*	*0.011*
Fat mass	**−0.330**	−0.164	−0.229
	0.019	*0.260*	*0.118*
EBW	**−0.512**	**−0.409**	−0.274
	<0.001	*0.004*	*0.060*
VAI	**−0.354**	−0.268	−0.227
	0.012	*0.062*	*0.121*
Insulin	**−0.351**		−0.054
	0.013		*0.713*
Triglycerides	**−0.311**	−0.242	−0.240
	0.028	*0.094*	*0.100*
Total cholesterol	**−0.285**	−0.234	−0.221
	0.045	*0.106*	*0.131*
LDL cholesterol	**−0.441**	**−0.298**	−0.226
	0.001	*0.038*	*0.122*
DBP	**−0.312**	−0.187	−0.008
	0.027	*0.193*	*0.550*
Peak GH	**0.445**	**0.306**	
	0.001	*0.032*	
IGF-1 SDS	**0.548**	**0.461**	
	<0.001	*0.001*	

Simple correlation coefficients are reported in first column, while partial correlation coefficients obtained after adjusting for fasting insulin or peak GH and IGF-1, respectively, are reported in the second and third columns. To correct for skewed distributions, Sirt4 and peak GH values were logarithmically transformed and back-transformed for presentation in the table. Significant correlations and their p values have been reported as bold-faced and italicized data, respectively.
BMI, Body Mass Index; *VAI*, Visceral adiposity index; *EBW*, Excess Body Weight; *DPB*, diastolic blood pressure; *GH*, growth hormone; *IGF-1*, insulin-like growth factor-1; *IGF-1 SDS*, IGF-1 z score or standard deviation score.
[a] Correlation coefficients were adjusted for insulin.
[b] Correlation coefficients were adjusted for GH status (peak GH and IGF-1 SDS).

4. Discussion

Our present results indicate that low serum Sirt4 levels in obese subjects are correlated to low peak GH and low IGF-1 levels. In addition, serum Sirt4 shows a negative correlation with anthropometric variables, such as BMI and waist circumference, with cardiovascular risk factors, i.e., insulin, triglycerides, total cholesterol, LDL cholesterol, DBP and VAI, recently considered a reliable indicator of cardio-metabolic risk [20].

This observation confirms and extends our previous finding that circulating levels of Sirt4 are low in obese subjects with ectopic fat storage in the liver and skeletal muscle and are associated with increased cardiovascular risk factors [16]. Although there is a limitation in its interpretation due to the small size of this cohort, we found in the present study that the subset of obese subjects with low peak GH and low IGF-1 levels have lower serum Sirt4 levels and higher BMI, waist circumference and VAI compared with their counterpart with normal GH/IGF-1 status. Thus, the question arises as to whether the low serum Sirt4 levels in the subset of obese subjects with low GH/IGF-1 status are only the consequence of their greater severity of obesity. Indeed, after adjusting the correlations between Sirt4 and anthropometric variables for peak GH and IGF-1, the relationship between Sirt4 and BMI became not significant. This preliminary result lets us hypothesize that GH/IGF-1 status was more powerful in influencing the Sirt4 levels. In addition, at the

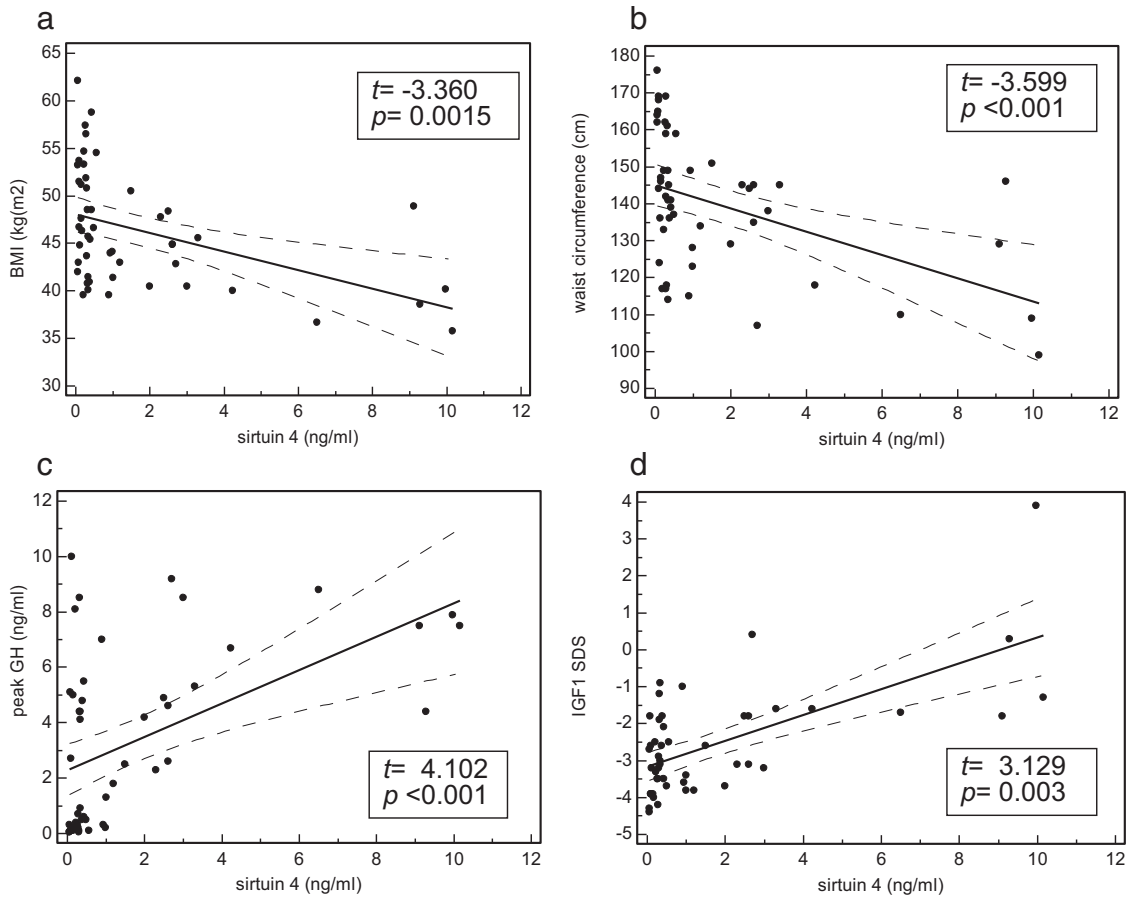

Fig. 2. Scatter diagrams & regression lines show the significant prediction of anthropometric variables, peak GH and IGF-1 levels as independent variables on serum Sirt4. The prediction of lower Sirt4 levels by major severity of BMI (a), higher waist circumference (b) and lower peak GH (c) and IGF-1 SDS (d) was well documented. Sirt4 and peak GH values were logarithmically transformed and back-transformed for presentation in the figure. *Sirtuin 4*, Sirt4; *BMI*, Body Mass Index; *GH*, growth hormone; *IGF-1*, insulin-like growth factor-1; *IGF-1 SDS*, IGF-1 z score or standard deviation score.

multiple regression analysis, between the anthropometric variables used in our series of obese subjects, peak GH and IGF-1, IGF-1 remained the major predictor for Sirt4.

In the clinical setting of the heterogeneity of obesity phenotype, it is currently accepted that the GH/IGF-1 status might play a pivotal role in contributing to determine different body compositions and cardiometabolic risk profiles among equally obese subjects [6–9]. Very recently, Stanley [24] reported that obese subjects presenting with reduction of either IGF-1 or peak GH had higher carotid intima-media thickness, a functional and structural marker of early atherosclerosis, compared to obese subjects with both normal peak GH and IGF-1 levels. GH and IGF-1, through their receptors expressed on skeletal muscle, have been reported to actively up-regulate mitochondrial oxidative capacity [3,4]. Thus, the low GH/IGF-1 status in obese individuals might represent the major contributor to reducing the β-oxidation as an example of the maladaptive endocrine changes operating in obesity.

Table 3
Multiple regression analysis model.

	R^2	β	t	p
IGF-1 SD	0.407	0.637	5.730	<0.001
Variables excluded				
Waist circumference		−0.220	−1.827	−0.074
Fasting insulin		0.027	−0.214	0.831
Peak GH		0.149	1.207	0.233

The multiple regression analysis (stepwise method) was used to estimate the predictive value of peak GH, IGF-1 SDS and waist circumference with Sirt4 as dependent variable. In this analysis, we entered only those variables that had a p-value <0.05 in the univariate analysis (partial correlation). The multiple regression analysis shows that IGF-1 was independently related to Sirt4, while waist circumference and peak GH lost their independent predictive power, highlighting the close relationship between low IGF-1 and low serum Sirt4.
Sirtuin 4, Sirt4; *GH*, growth hormone; *IGF-1*, insulin-like growth factor-1; *IGF-1 SDS*, IGF-1 z score or standard deviation score.

In addition, a role for Sirt4 in regulating fat metabolism by increasing β-oxidation of fatty acid has been well described, due to its ability to deacetylate malonyl-CoA-decarboxylase. In particular, Sirt4 inhibition increases fat oxidative capacity in the liver and mitochondrial function in muscle, with possible therapeutic benefits for diseases associated with ectopic lipid storage such as T2DM [25]. Moreover, in Sirt4 knockout mice, Sirt4 deficiency leads to a protection from high fat diet-induced obesity linked to the increased β-oxidation [26]. Finally, fasting and/or calorie restriction are reported to be associated with reduced expression of hepatic Sirt4 [25]. Thus, the GH/IGF-1 axis and Sirt4 serve as active regulators of mitochondrial oxidative capacity with opposite functions. A number of recent studies have indicated the possible implication of impaired skeletal muscle mitochondrial oxidative phosphorylation as the underlying etiologic element of a variety of disorders including obesity, insulin resistance, and diabetes [27,28], although the causative or compensatory effect of mitochondrial oxidative capacity remained to be elucidated [29].

The precise pathophysiological mechanisms linking low serum Sirt4 with low GH/IGF-1 status were not thoroughly analyzed in this study as the comparative tissue evaluation of Sirt4 expression or activity in cell cultures was lacking. However, for ethical issues we preferred to avoid performing muscle or liver biopsies as preliminary approach in this study. Nevertheless, the association between low Sirt4, low peak GH

and low IGF-1 levels in the clinical setting of obesity and cardiovascular risk could have an intriguing hypothesis. In our previous work we suggested that low serum Sirt4, likely mirroring a reduced mitochondrial expression, represented an attempt to preserve in obesity the mitochondrial oxidative capacity, providing benefits for the associated ectopic lipid storage excess. In the present study, vice versa, it is tempting to speculate that in obese patients, low GH/IGF-1 status, most frequently characterized by a higher prevalence of cardio-metabolic risk factors, is likely associated with a major compensatory decrease in circulating levels of Sirt4 to oppose to its negative regulator effect on mitochondrial oxidative capacity. A clinical implication is that the concomitant evaluation of circulating levels of Sirt4 in addition to other classic risk factors in obese individuals could help to better characterize the subset of subjects with low GH/IGF-1 status, higher cardio-metabolic risk and lower effective adaptation to calorie restriction.

Aside from obesity, glucose metabolism is another factor which might influence Sirt4. Evidence has been provided that mRNA expression of Sirt4 in granulocytes and monocytes of T2DM patients was lower compared with healthy individuals, with a negative correlation between Sirt4 and fasting plasma glucose [12]. However, after adjusting for insulin, Sirt4 still remained significantly correlated with BMI, peak GH and IGF-1 levels.

We are aware that there are some limitations in our study. First, our sample size is small. Second, the cross-sectional design of the study precludes the possibility of establishing the direction of causality between low Sirt4 with low GH/IGF-1 status in obesity. Third, we do not provide experimental data on muscle biopsy sample to confirm the link between Sirt4 and GH/IGF-1 status; thus, our hypothesis of a compensative reduction of serum Sirt4 as the consequence of the low GH/IGF-1 status remains largely speculative.

In conclusion, there was a close relationship between low serum Sirt4 and low IGF-1, likely representing a compensatory mechanism to oppose to the negative regulator effect exerted by Sirt4 on mitochondrial oxidative capacity. Further studies on larger series of obese subjects will help confirm if the relationship between Sirt4 and GH/IGF-1 status is only a matter of an adaptive mechanism to the obesity-related reduced mitochondrial oxidative capacity or is a dependent effect of the perturbations along the GH/IGF-1 axis.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

[1] A. Vijayakumar, S. Yakar, D. LeRoith, The intricate role of growth hormone in metabolism, Front. Endocrinol. (Lausanne) 2 (2011) 32–43.
[2] A. Vijayakumar, Y. Wu, N.J. Buffin, X. Li, H. Sun, R.E. Gordon, S. Yakar, D. LeRoith, Skeletal muscle growth hormone receptor signaling regulates basal, but not fasting-induced, lipid oxidation, PLoS One 7 (2012) e44777.
[3] K.R. Short, N. Moller, M.L. Bigelow, J. Coenen-Schimke, K.S. Nair, Enhancement of muscle mitochondrial function by growth hormone, J. Clin. Endocrinol. Metab. 93 (2008) 597–604.
[4] A. Garten, S. Schuster, W. Kiess, The insulin-like growth factors in adipogenesis and obesity, Endocrinol. Metab. Clin. North Am. 41 (2012) 283–295.
[5] D.E. Berryman, C.A. Glad, E.O. List, G. Johannsson, The GH/IGF-1 axis in obesity: pathophysiology and therapeutic considerations, Nat. Rev. Endocrinol. 9 (2013) 346–356.
[6] F. Cordido, J. Garcia-Buela, S. Sangiao-Alvarellos, T. Martinez, O. Vidal, The decreased growth hormone response to growth hormone releasing hormone in obesity is associated to cardiometabolic risk factors, Mediat. Inflamm. 2010 (2010) 434–562.
[7] M.A. Bredella, A.L. Utz, M. Torriani, B. Thomas, D.A. Schoenfeld, K.K. Miller, Anthropometry, CT, and DXA as predictors of GH deficiency in premenopausal women: ROC curve analysis, J. Appl. Physiol. 106 (2009) 418–422 ((1985)).
[8] S. Savastano, C. Di Somma, A. Belfiore, B. Guida, F. Jr Orio, F. Rota, M.C. Savanelli, T. Cascella, A. Mentone, L. Angrisani, G. Lombardi, A. Colao, Growth hormone status in morbidly obese subjects and correlation with body composition, J. Endocrinol. Investig. 29 (2006) 536–543.
[9] C. Di Somma, R. Pivonello, G. Pizza, A. De Rosa, G. Lombardi, A. Colao, S. Savastano, Prevalence of the metabolic syndrome in moderately–severely obese subjects with and without growth hormone deficiency, J. Endocrinol. Investig. 33 (2010) 171–177.
[10] M.C. Haigis, D.A. Sinclair, Mammalian sirtuins: biological insights and disease relevance, Annu. Rev. Pathol. 5 (2010) 253–295.
[11] N. Ahuja, B. Schwer, S. Carobbio, D. Waltregny, B.J. North, V. Castronovo, P. Maechler, E. Verdin, Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase, J. Biol. Chem. 282 (2007) 33583–33592.
[12] R. Song, W. Xu, Y. Chen, Z. Li, Y. Zeng, Y. Fu, The expression of Sirtuins 1 and 4 in peripheral blood leukocytes from patients with type 2 diabetes, Eur. J. Histochem. 55 (2011) e10.
[13] E. Verdin, M.D. Hirschey, L.W. Finley, M.C. Haigis, Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling, Trends Biochem. Sci. 35 (2010) 669–675.
[14] G. Tarantino, S. Savastano, A. Colao, Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance, World J. Gastroenterol. 16 (2010) 4773–4783.
[15] G. Medina-Gómez, Mitochondria and endocrine function of adipose tissue, Best Pract. Res. Clin. Endocrinol. Metab. 26 (2012) 791–804.
[16] G. Tarantino, C. Finelli, F. Scopacasa, F. Pasanisi, F. Contaldo, D. Capone, S. Savastano, Circulating levels of Sirtuin 4, a potential marker of oxidative metabolism, related to coronary artery disease in obese patients suffering from NAFLD, with normal or slightly increased liver enzymes, Oxidative Med. Cell. Longev. (2014), http://dx.doi.org/10.1155/2014/920676.
[17] American Diabetes Association, Standards of medical care in diabetes—2014, Diabetes Care 37 (2014) S17–S80.
[18] A. Colao, C. Di Somma, T. Cascella, R. Pivonello, G. Vitale, L.F. Grasso, G. Lombardi, S. Savastano, Relationships between serum IGF1 levels, blood pressure, and glucose tolerance: an observational, exploratory study in 404 subjects, Eur. J. Endocrinol. 159 (2008) 389–397.
[19] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.
[20] M.C. Amato, C. Giordano, M. Galia, A. Criscimanna, S. Vitabile, M. Midiri, A. Galluzzo, AlkaMeSy Study Group, Visceral Adiposity Index: a reliable indicator of visceral fat function associated with cardiometabolic risk, Diabetes Care 33 (2010) 920–922.
[21] E. Ghigo, G. Aimaretti, L. Gianotti, J. Bellone, E. Arvat, F. Camanni, New approach to the diagnosis of growth hormone deficiency in adults, Eur. J. Endocrinol. 134 (1996) 352–356.
[22] G. Corneli, C. Di Somma, R. Baldelli, S. Rovere, V. Gasco, C.G. Croce, S. Grottoli, M. Maccario, A. Colao, G. Lombardi, E. Ghigo, F. Camanni, G. Aimaretti, The cut-off limits of the GH response to GH-releasing hormone–arginine test related to body mass index, Eur. J. Endocrinol. 153 (2005) 257–264.
[23] M.E. Molitch, D.R. Clemmons, S. Malozowski, G.R. Merriam, M.L. Vance, Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline, J. Clin. Endocrinol. Metab. 96 (2011) 1587–1609.
[24] T.L. Stanley, M.N. Feldpausch, C.A. Murphy, S.K. Grinspoon, H. Makimura, Discordance of IGF-1 and GH stimulation testing for altered GH secretion in obesity, Growth Hormon. IGF Res. 24 (2014) 10–15.
[25] N. Nasrin, X. Wu, E. Fortier, Y. Feng, O.C. Bare, S. Chen, X. Ren, Z. Wu, R.S. Streeper, L. Bordone, SIRT4 regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells, J. Biol. Chem. 285 (2010) 31995–32002.
[26] G. Laurent, N.J. German, A.K. Saha, V.C. de Boer, M. Davies, T.R. Koves, N. Dephoure, F. Fischer, G. Boanca, B. Vaitheesvaran, S.B. Lovitch, A.H. Sharpe, I.J. Kurland, C. Steegborn, S.P. Gygi, D.M. Muoio, N.B. Ruderman, M.C. Haigis, SIRT4 coordinates the balance between lipid synthesis and catabolism by repressing malonyl CoA decarboxylase, Mol. Cell 50 (2013) 686–698.
[27] M. Liesa, O.S. Shirihai, Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure, Cell Metab. 17 (2013) 491–506.
[28] J.R. Peinado, A. Diaz-Ruiz, G. Frühbeck, M.M. Malagon, Mitochondria in metabolic disease: getting clues from proteomic studies, Proteomics 4–5 (2014) 452–466.
[29] P. Schrauwen, M.K. Hesselink, Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes, Diabetes 53 (2004) 1412–1417.