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Circulating SIRT1 inversely correlates with epicardial fat thickness in patients with obesity

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Abstract *Background and aim:* Obesity is increasing worldwide and is related to undesirable cardiovascular outcomes. Epicardial fat (EF), the heart visceral fat depot, increases with obesity and correlates with cardiovascular risk. SIRT1, an enzyme regulating metabolic circuits linked with obesity, has a cardioprotective effect and is a predictor of cardiovascular events. We aimed to assess the relationship of EF thickness (EFT) with circulating SIRT1 in patients with obesity.
Methods and results: Sixty-two patients affected by obesity and 23 lean controls were studied. Plasma SIRT1 concentration was determined by enzyme-linked immunosorbent assay (ELISA). EFT was measured by echocardiography. Body mass index (BMI), waist circumference, heart rate (HR), blood pressure, and laboratory findings (fasting glucose, insulin, HbA1c, cholesterol, and triglycerides) were assessed.

SIRT1 was significantly lower ($P = 0.002$) and EFT was higher ($P < 0.0001$) in patients with obesity compared with lean controls. SIRT1 showed a negative correlation with EFT and HR in the obesity group ($\rho = -0.350$, $P = 0.005$; $\rho = -0.303$, $P = 0.008$, respectively). After adjustment for obesity-correlated variables, multiple linear regression analysis showed that EFT remained the best correlate of SIRT1 ($\beta = -0.352$, $P = 0.016$).
Conclusions: Circulating SIRT1 correlates with the visceral fat content of the heart. Serum SIRT1 levels might provide additional information for risk assessment of coronary artery disease in patients with obesity.

Introduction

Sirtuins (SIRTs) regulate many metabolic adaptations in obesity [1]. SIRT1, the most intensely studied sirtuin family member, regulates the expression of adipokines, represses the activity of factors required for maturation of fat cells, alters mitochondrial capacity, regulates insulin secretion and sensitivity, and modulates plasma glucose levels [1]. In addition, SIRT1 prevents diet-induced obesity and associated non-alcoholic fatty liver disease [2], a condition of ectopic fat accumulation accompanied by a decrease in SIRT1 expression at both circulating [3] and visceral adipose tissue (VAT) levels [4]. In line with these observations, the weight loss induces a SIRT1 concentration increase in plasma [5], adipose tissue, and liver [6] in patients with obesity. Overall, SIRT1 is found to be protective against endothelial dysfunction, atherothrombosis, myocardial infarction [2], and cardiovascular diseases in general. Indeed, by controlling the cardiac metabolic gene expression through modulation of the peroxisome

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proliferator activated receptor α (PPARα) pathway, SIRT1 protects the heart from hypertrophy, metabolic dysregulation, and inflammation [7,2].

Epicardial fat (EF), the adipose tissue of the heart, is a VAT. It covers 80% of the heart's surface and, because of its anatomical contiguity with the heart, locally modulates the functions of myocardium and coronary arteries through a paracrine cross talk [8–10]. EF has potential cardioprotective effects that can be exerted mechanically, thermogenically, and throughout the local secretion of adipokines [8]. However, excess EF carries a great prediction of mortality [11]. Specifically, EF thickness (EFT) is associated with premature coronary artery disease [12] and obstructive sleep apnea [13]. EFT increases in prediabetic patients, regardless of fasting plasma glucose and HbA1c [14], and in patients with type 2 diabetes mellitus and obesity [15], representing a diagnostic criterion for patients with metabolic syndrome [16]. It is also inversely correlated with endothelial function [17]. In this study, we focused on the association of circulating SIRT1 levels with the visceral fat of the heart, represented by EF, in patients affected by obesity.

Methods

Study population

We studied 85 subjects, 62 affected by obesity (19 men and 43 women) and 23 healthy lean controls (7 men and 19 women). Study participants were recruited from among subjects referred to the High Specialization Center for the Care of Obesity at the Department of Experimental Medicine, "Sapienza" University of Rome. Inclusion criteria comprised age (18–65 years), race (Caucasian ethnicity), BMI $\geq$ 30 kg/m^2 for obese patients, and BMI $\geq$ 18.5 and $\leq$ 24.9 kg/m^2 for controls. As exclusion criteria, we considered corticosteroids for systemic use, any medication, or any clinical condition potentially affecting body weight, autoimmune diseases, renal failure, heart failure, type 1 diabetes, and malignant disease during the last 5 years.

The average age was 39.58 $\pm$ 12.27 years (range 18–65 years) for subjects with obesity and 41.69 $\pm$ 10.84 years (range 22–59 years) for controls. Subjects affected by obesity and previously diagnosed with diabetes mellitus, dyslipidemia, or hypertension were receiving glucose-, lipid-, and BP-lowering agents for each of these conditions. All subjects were enrolled after written informed consent. The study was approved by the local ethical committee and was concordant with the Helsinki Declaration.

Measurements

The patients underwent complete medical examination and anthropometric measurements (body weight, height, waist circumference (WC)). Body weight was measured by Tanita BWB-800A digital medical scale (Tanita Corporation, Arlington Heights, IL, USA). Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). After overnight fasting, serum SIRT1, glycemia, insulin, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides were determined. Heart rate (HR) and systolic and diastolic blood pressure (mmHg) measured by a mercury sphygmomanometer (Riva-Rocci System, ERKA, Chemnitz, Germany) were recorded. EFT (mm) was measured by ultrasound.

SIRT1 assays

The plasma SIRT1 concentration was determined by a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) method, using a commercially available human SIRT1 ELISA kit (MyBioSource, Cod. GDMBS 705558) as previously described [3]. Microtiter plates were coated with equal amount of primary mouse anti-human SIRT1 monoclonal IgG. Hundred-microliter standard and serum samples were pipetted in each well and the protocol was followed by using secondary avidin conjugated with horseradish peroxidase. The formation of horseradish peroxidase was measured at 405 nm using an ELISA reader (Quanta Biotech, UK). Seven different concentrations of purified SIRT1 (0.15, 0.312, 0.625, 1.25, 2.5, 5.0, and 10 ng/mL) were used to plot a standard curve. The inter- and intra-assay coefficients of variation were 4% and 6%, respectively, with a detection limit of 0.1 ng/mL.

Epicardial fat thickness measurements

EFT was measured through a validated echocardiographic procedure [18]. Participants underwent high-resolution M-B-mode transthoracic echocardiography using a 2.5-MHz probe and spectral Doppler exam of the common carotid artery using a 7.5-MHz probe (Esaote MyLab40, Esaote Europe B.V., the Netherlands). The EFT was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium, and its thickness was measured perpendicularly on the free wall of the right ventricle (RV) at the end systole in three cardiac cycles. The average value of three cardiac cycles from each echocardiographic view was considered. All echocardiograms were recorded by the same experienced operator, who was blinded to the other study data.

Statistical analysis

Results were expressed as mean $\pm$ SD. The degree of association between variables was calculated using Spearman's nonparametric correlation. A p-value of <0.05 was considered statistically significant. Sex- and age-adjusted multivariate regression analyses were realized in subjects with obesity to verify the associations among SIRT1 and relevant variables (EFT, HR, BMI, WC, and HbA1c). Data were analyzed with the use of STATISTICA software, version 6.1 (StatSoft, Inc., Tulsa, OK, USA).

Results

The demographic, anthropometric, and clinical characteristics of the patients are shown in Table 1. The patients with obesity (BMI, mean ± SD 41.82 ± 7.67 kg/m^2) showed echocardiographic evidence of increase in EFT ($P < 0.0001$) and significantly lower SIRT1 circulating levels ($P = 0.002$) compared with normal-weight subjects. The WC was constantly ≥80 cm in females and ≥94 cm in males with obesity. The group of healthy lean controls (BMI, mean ± SD 23.26 ± 1.60 kg/m^2) had no ultrasound evidence of increase in EFT. The total cholesterol ($P = 0.045$), LDL-C ($P = 0.040$), systolic BP ($P = 0.041$), diastolic BP ($P = 0.011$), and insulin ($P = 0.020$) levels were significantly lower in the population of lean controls (Table 1).

The correlation between SIRT1 and the other variables was analyzed using the Spearman correlation coefficient test. In the population with obesity, SIRT1 was negatively correlated with EFT ($\rho = -0.350$; $P = 0.005$) (see Fig. 1) and HR ($\rho = -0.303$; $P = 0.008$). On the contrary, SIRT1 did not correlate with BMI ($P = 0.326$), WC ($P = 0.263$), FPG ($P = 0.415$), and the other parameters tested. A tendency to a negative correlation with HbA1c was also seen ($\rho = -0.216$; $P = 0.062$). In the healthy lean control group, SIRT1 did not correlate with any of the parameters measured (data not shown).

Sex- and age-adjusted multivariate regression analyses for the associations between SIRT1 and other relevant variables (EFT, HR, BMI, WC, and HbA1c) in patients with obesity are shown in Table 2. The analysis revealed a significant negative correlation between the levels of SIRT and the EFT, which remained the best independent correlate of SIRT1 ($R = 0.429$, $R^2 = 0.184$, $\beta = -0.352$, $P = 0.016$).

Table 1 Demographic, anthropometric, and clinical characteristics of the patients.

Variables	Obese subjects ($n = 62$)	Normal weight ($n = 23$)	P
Age (years)	39.58 ± 12.27	41.69 ± 10.84	0.439
SIRT1 (ng/ml)	1.36 ± 1.31	2.27 ± 1.13	0.002
Weight (kg)	116.17 ± 24.36	65.09 ± 7.07	<0.0001
BMI (kg/m^2)	41.82 ± 7.67	23.26 ± 1.60	<0.0001
WC (cm)	126.21 ± 14.71	78.80 ± 11.91	<0.0001
Epicardial fat (mm)	8.49 ± 0.87	6.97 ± 0.57	<0.0001
Heart rate (bpm)	73.58 ± 8.53	74.30 ± 6.44	0.695
FPG (mg/100 ml)	100.57 ± 21.42	98.65 ± 16.15	0.677
Insulin (mcUI/ml)	16.48 ± 14.04	9.88 ± 3.95	0.020
HbA1c (%)	5.05 ± 0.62	4.77 ± 0.92	0.089
Systolic BP (mmHg)	127.36 ± 14.51	120.57 ± 14.09	0.041
Diastolic BP (mmHg)	79.34 ± 10.46	73.57 ± 7.43	0.011
Total-C (mg/100 ml)	197.69 ± 33.45	183.42 ± 21.92	0.045
LDL-C (mg/100 ml)	120.68 ± 28.17	107.66 ± 25.71	0.040
HDL-C (mg/100 ml)	48.66 ± 12.90	49.30 ± 15.22	0.835
Triglycerides (mg/100 ml)	132.81 ± 82.86	108.36 ± 50.61	0.160

Abbreviations: SIRT1, sirtuin1; BMI, body mass index; WC, waist circumference; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; BP, blood pressure; HDL-C, high-density lipoprotein-cholesterol; LDL-C, Low-density lipoprotein-cholesterol. Values are expressed as means ± SD.

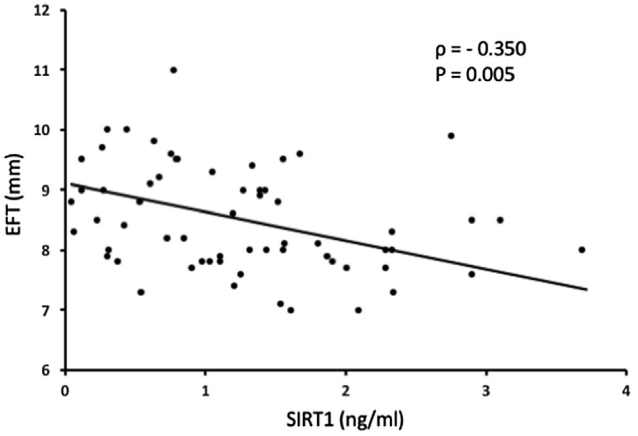

Figure 1 Correlation between circulating SIRT1 and epicardial fat thickness in people with obesity. Correlation coefficient (ρ) and level of significance (P) are provided.

According to previous studies [19], a correlation between EFT and both BMI ($\rho = 0.617$, $P < 0.0001$) and WC ($\rho = 0.640$, $P < 0.0001$) was found.

Discussion

VAT, including the EF surrounding the heart, shows many histological and functional peculiarities. VAT contains a large number of inflammatory cells and expresses several hormone receptors, and its adipocytes are metabolically active and sensitive to lipolysis. The increased amount of VAT carries, in general, a great prediction of mortality [11] and EF, which substantially mirrors visceral adiposity rather than general obesity, has been correlated with cardiovascular risk factors [8,13,14]. EFT increases in obese patients. It is a useful parameter in the assessment of patients with obesity and other cardio-metabolic diseases [17]. Due to its proximity to the heart, by secreting pro-inflammatory cytokines EF exerts direct local metabolic effects. This condition promotes local cardiovascular diseases and contributes, together with other ectopic fat depots, to metabolic derangements and diabetes mellitus [20].

SIRT1, a (NAD+)-dependent enzyme, is significantly involved in the metabolic adaptations observed in obesity

Table 2 Age- and sex-adjusted multivariate regression analysis of SIRT1.

Independent variables	β	p
EFT (mm)	−0.352	0.016
HR (bpm)	−0.133	0.316
BMI (kg/m^2)	0.134	0.557
WC (cm)	−0.096	0.689
HbA1c (%)	−0.157	0.220

Abbreviations: SIRT1, sirtuin1; EFT, epicardial fat thickness; HR, heart rate; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin. EFT, HR, BMI, WC, and HbA1c were entered in the multiple regression analysis to predict SIRT1, the dependent variable. EFT was the best independent correlate of SIRT1 in patients with obesity.

[1]. Most studies report low SIRT1 in subjects affected by obesity and higher SIRT1 expression after weight loss, both in tissues [6] and in bloodstream [5], suggesting that SIRT1 is downregulated in obesity. The awareness of the role of SIRT1 against metabolic derangements such as diabetes, dyslipidemia, and liver steatosis has been increasing [21, [22]. This role is further highlighted by the observation of a low SIRT1 pathway expression in subcutaneous adipose tissue from a population of BMI-discordant monozygotic twins [23] affected by obesity, inflammation, insulin resistance, and impaired mitochondrial protein homeostasis. This datum, besides suggesting a strong relationship of reduced SIRT1 expression with indices of metabolic dysfunction, indicates a close relationship between SIRT1 and acquired obesity independent of the genetic background [23].

To date, substantial evidence suggests a role of SIRT1 in cardioprotection [2]. The relevance of SIRT1 as a cardiac gene regulator is well established, and low SIRT1 expression in the context of cardiovascular disease has been reported [7]. Furthermore, SIRT1 shows a significant contribution in oxidant and antioxidant balance in heart failure [24] and, via activating eNOS, its overexpression protects against myocardial ischemia–reperfusion injury in diabetic rats, representing a promising therapeutic target for cardiac complications in diabetics [25]. Finally, the SIRT1 gene KO leads to cardiac malformations and increased perinatal mortality in mice [26].

In this study, we evaluated the relationship between SIRT1 and the visceral fat depot of the heart. We found that EFT is a strong negative correlate of the circulating SIRT1 in individuals affected by obesity. This behavior closely resembles that of other bioactive compounds produced by EF with reported cardioprotective effects, both in vivo and in vitro. For example, the increase in EFT is accompanied by a reduced expression of adiponectin [27], decreased circulating adiponectin levels in genetic and diet-induced murine models of obesity, and downregulated EF adrenomedullin gene and protein expression in subjects with coronary artery disease [28]. Thus, SIRT1, analogously to bioactive adipokines originating from EF, shows a correlation with cardio-metabolic status and amount of EF. Accordingly, EF revealed a unique transcriptome that shows profound modifications in patients with coronary artery disease [29], and among the differentially expressed genes in human EF to identify molecules associated with cardiovascular diseases, SIRT1 was represented [30]. Consistent with previous studies [3,5], we did not find a relationship between circulating SIRT1 and BMI or WC, although an association among EFT, BMI, and WC was seen. This observation confirms that the increase in BMI and WC may not reflect a reduction in SIRT1, which is a distinctive trait of individuals mostly with visceral obesity, nor may it be representative of the excess visceral fat depot responsible for the severity of cardio-metabolic complications of obesity. Furthermore, since regional fat depots may be of greater importance than overall adiposity, traditional anthropometric measures should be viewed with caution. Our finding highlights the poor relationship between SIRT1 and the classic measures of adiposity. Indeed, analogously to the recent observation of a negative association between SIRT1 and liver steatosis [3], a condition of ectopic fat accumulation, we found a significant correlation between SIRT1 and EF, the ectopic visceral fat depot of the heart whose major drive is not obesity but excessive visceral fat accumulation [8]. Thus, SIRT1 is preferentially associated with visceral fat rather than with obesity per se. From this point of view, SIRT1 could be elevated to the rank of marker associated with the predisposition to accumulate fat viscerally. It is noteworthy that in normal-weight patients, who had higher SIRT1 concentration and lower EFT than patients with obesity did, we observed that the correlation between SIRT1 and EFT was lacking. Interestingly, myocardial dysfunction seems to be related to scarce metabolic control rather than to fat mass or BMI [31]. Previous data have suggested that patients with obesity yet metabolically healthy carry a reduced risk of cardiovascular disease or mortality than patients with normal weight yet metabolically unhealthy [32]. However, the definition of metabolically healthy obesity is established using BMI, an improper index of adiposity. Therefore, SIRT1 measurement could help in stratifying cardiovascular disease risk and identifying metabolically healthy or unhealthy obese phenotypes. The exact mechanisms involved in the inverse relationship between circulating SIRT1 and EFT reported here are unknown. Mounting evidence shows that SIRT transcription and/or protein levels are persistently reduced in specific tissue during chronic inflammation. Examples include fat deposits in obesity with inflammation, brain in Alzheimer's disease, and arterial inflammation in atherosclerosis [33]. Thus, a working hypothesis, which deserves further investigation, is that excess EF in patients with visceral obesity displays a pro-inflammatory phenotype; an oxidative stress environment is created, mainly due to a state of chronic inflammation prone to secreting tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and inducible nitric oxide synthase (iNOS), with heightened inflammation associated with low SIRT1 and expansion of adipose tissue.

Although EF may be a major source of circulating SIRT1, because of the widespread distribution of the potential tissue sources of SIRT1 [21], additional sources of circulating SIRT1, besides EF, cannot be ruled out.

Unexpectedly, SIRT1 correlated with the HR in patients with obesity. The exact mechanism for such an association is unclear, and this aspect of the study deserves further investigation. However, the diversity of SIRT1 targets may explain the complexity of its function in different tissues, including the cardiac electric tissue. Consistently, increased SIRT1 expression is found in the right auricle tissues of patients with atrial fibrillation (AF) [34]. Moreover, EF is significantly thicker in individuals with chronic AF than in those with paroxysmal AF, suggesting that long-term exposure to EF excess might relate to chronic AF [35].

Finally, recent data showed that PDE5 inhibitor treatment in humans reduces EF and upregulates SIRT1 in both serum and subcutaneous fat, opening novel therapeutic

strategies for EF remodeling and regulation of SIRT1 to promote healthier fat deposits [36].

Conclusion

In conclusion, the findings of this study suggest that in people with obesity, SIRT1, a metabolically crucial enzyme, is inversely and independently associated with EFT, but no relationship between circulating SIRT1 and BMI or WC was seen. A large body of evidences suggests a role of SIRT1 as a predictor of cardiovascular events, and our present data seem to go in the same direction. Overall, the down-regulation of SIRT1 expression observed at the circulating level and in visceral and subcutaneous adipose tissues of people with obesity [37], paralleled by the increase in epicardial VAT, may contribute to the cardio-metabolic abnormalities associated with obesity. This field will be further investigated in the light of the disposability of SIRT1 mimetics/activators able to increase SIRT1 activities.

Disclosures

The authors declared no conflict of interest.

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