Endocrine
DOI 10.1007/s12020-016-1170-8

ORIGINAL ARTICLE

Sirtuins 1–7 expression in human adipose-derived stem cells from subcutaneous and visceral fat depots: influence of obesity and hypoxia

Stefania Mariani[1] · Giuliana Di Rocco[2] · Gabriele Toietta[2] · Matteo A. Russo[3] · Elisa Petrangeli[1] · Luisa Salvatori[4]

Received: 17 January 2016 / Accepted: 3 November 2016

Abstract The sirtuin family comprises seven NAD^+-dependent deacetylases which control the overall health of organisms through the regulation of pleiotropic metabolic pathways. Sirtuins are important modulators of adipose tissue metabolism and their expression is higher in lean than obese subjects. At present, the role of sirtuins in adipose-derived stem cells has not been investigated yet. Therefore, in this study, we evaluated the expression of the complete panel of sirtuins in adipose-derived stem cells isolated from both subcutaneous and visceral fat of non-obese and obese subjects. We aimed at investigating the influence of obesity on sirtuins' levels, their role in obesity-associated inflammation, and the relationship with the peroxisome proliferator-activated receptor delta, which also plays functions in adipose tissue metabolism. The mRNA levels in the four types of adipose-derived stem cells were evaluated by quantitative polymerase chain reaction, in untreated cells and also after 8 h of hypoxia exposure. Correlations among sirtuins' expression and clinical and molecular parameters were also analyzed. We found that sirtuin1–6 exhibited significant higher mRNA expression in visceral adipose-derived stem cells compared to subcutaneous adipose-derived stem cells of non-obese subjects. Sirtuin1–6 levels were markedly reduced in visceral adipose-derived stem cells of obese patients. Sirtuins' expression in visceral adipose-derived stem cells correlated negatively with body mass index and C-reactive protein and positively with peroxisome proliferator-activated receptor delta. Finally, only in the visceral adipose-derived stem cells of obese patients hypoxia-induced mRNA expression of all of the sirtuins. Our results highlight that sirtuins' levels in adipose-derived stem cells are consistent with protective effects against visceral obesity and inflammation, and suggest a transcriptional mechanism through which acute hypoxia up-regulates sirtuins in the visceral adipose-derived stem cells of obese patients.

Keywords Sirtuins · Adipose-derived stem cells · Obesity · Hypoxia · Inflammation · Peroxisome proliferator-activated receptor delta

Introduction

Sirtuins (SIRTs) are NAD^+-dependent class III histone deacetylases [1], which play important roles in modulating metabolic processes, are central to the control of energy homeostasis and deeply involved in adipose tissue (AT) metabolism [2]. SIRT1 is an important modulator of AT differentiation and remodeling. Indeed, SIRT1 inhibits adipogenesis and promotes fat mobilization by repressing activity of the peroxisome proliferator-activated receptor (PPAR) γ [3], induces browning of white AT promoting energy expenditure over energy storage [4], activates lipolysis [5], improves insulin signaling [6], and increases the

✉ Luisa Salvatori
luisa.salvatori@uniroma1.it

1 Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy

2 Department of Research, Advanced Diagnostics, and Technological Innovation, Regina Elena National Cancer Institute, Rome, Italy

3 Consorzio MEBIC, San Raffaele University, Laboratory of Molecular and Cellular Pathology, Rome, Italy

4 Institute of Molecular Biology and Pathology, CNR, Rome, Italy

Published online: 14 November 2016

expression of adiponectin [7], which is inversely correlated with adiposity. Accordingly, the mRNA levels of SIRT1 are suppressed in AT of obese compared to normal-weight subjects [8, 9]. Although SIRT1 is the best known member of the family, the knowledge of the functions of the other less-described isoforms in AT is rapidly improving. SIRT2 has been shown to promote lipolysis and suppress adipocyte differentiation and adipogenesis through the inhibition of PPARγ [10, 11]; SIRT3 controls thermogenesis in brown AT, and its reduction correlates with obesity [12]; SIRT4 coordinates the balance between lipid synthesis and catabolism [13]; finally, SIRT6 has been shown to inhibit triglyceride biosynthesis [14].

Functions of peroxisome proliferator-activated receptor delta (PPARδ) in AT metabolism and weight control have also been uncovered. Indeed, PPARδ enhances fatty acid oxidation and energy uncoupling in AT, protects against diet-induced obesity and improves insulin sensitivity [15, 16].

The obese AT dysfunction is in part due to its hypoxic microenvironment and low-grade chronic inflammation [17]. SIRT1 appears to be protective from obesity-induced inflammation, as its reduction causes macrophage recruitment and AT inflammation during chronic high-fat feeding. Consistently, SIRT1 expression negatively correlates with AT macrophage content in lean and obese subjects [18]. Moreover, SIRT1 can repress inflammatory gene expression in both adipocytes and macrophages [6, 19]. Collectively, the above mentioned data suggest a decisive role of SIRTs against metabolic derangements and inflammation in obesity.

Recently, we analyzed the features of the adipose-derived stem cells (ASC), multipotent mesenchymal cells isolated from the stromal vascular fraction of the AT [20], and their contribution to obesity-associated inflammation [21, 22]. To date, there are no data on the role of SIRTs in ASC. Therefore, in this study we evaluated the expression of the seven mammalian SIRTs in both subcutaneous and visceral ASC from non-obese and obese subjects and highlighted their modulation in obesity. Moreover, the role of SIRTs in obesity-related inflammation as well as the interplay with PPARδ were shown.

Materials and methods

Patients' enrollment and ASC isolation and culture

ASC were obtained from both subcutaneous and visceral AT (SAT and VAT, respectively) of 12 individuals, 4 non-obese (2 males and 2 females, body mass index (BMI) $< 30\ kg/m^2$) and 8 obese (2 males and 6 females, BMI $> 30\ kg/m^2$). BMI, fasting plasma glucose (FPG), fasting insulin, glycated hemoglobin (HbA1c), total cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, fibrinogen and C-reactive protein (CRP) were recorded. SAT and VAT specimens were obtained as previously described [22]. Tissues were digested and the collected cells plated in complete Dulbecco's modified Eagle's medium [21]. In the experiments described below, ASC were usually used at the 5^{th} passage of in vitro culture. The study was approved by the Institutional Review Board of the Policlinico Umberto I in Rome, and was compliant with Helsinki Declaration. The patients gave their informed consent before the surgical procedure.

Cell culture in hypoxic conditions

To evaluate cell behavior during oxygen deprivation, the culture plates were incubated for 8 h in a hypoxia chamber (Billups-Rothenberg, Del Mar, CA) flushed with an atmosphere containing 1 % oxygen, 5 % CO_2 and balanced with nitrogen, as previously described in detail [22].

Quantitative PCR analysis

The mRNA expression level in the four types of ASC, cultured both in normoxic conditions and after exposure to hypoxia, was evaluated using TaqMan Array Microfluidic Cards designed with a purposely chosen panel of genes through the ABI Prism 7900HT Fast Real-Time PCR System Detector (Applied Biosystems, Foster City, CA) according to the manufacturer's default cycling conditions. The primer/probe sets used were as follows: SIRT1, Hs01009005_m1; SIRT2, Hs00247263_m1; SIRT3, Hs00202030_m1; SIRT4, Hs00202033_m1; SIRT5, Hs00202043_m1; SIRT6, Hs00213036_m1; SIRT7, Hs00213029_m1; 18S, Hs99999901_s1. Relative quantitation of the SIRTs' gene expression was performed using the $2^{-\Delta\Delta Ct}$ method, using the average of the four samples of subcutaneous ASC from non-obese subjects in basal conditions as calibrator. Human 18S ribosomal RNA was used as endogenous reference gene.

Statistical analysis

Data were analyzed by GraphPad Prism v5.01 (GraphPad Software, San Diego, CA) and are presented as means ± SEM. The paired *t*-test was used to evaluate the effect of hypoxic treatment with respect to untreated cells and to analyze the differences of clinical parameters in the two groups of individuals. One-way ANOVA with Bonferroni post-test for multiple comparisons was used to compare the ASC populations. Spearman correlation coefficient test was used to analyze the relationships between variables. P values < 0.05 were considered significant.

Table 1 Clinical characteristics of non-obese and obese patients

	Non-obese	Obese	*P*-value
Age (years)	52.00 ± 4.80	40.40 ± 1.14	0.178
BMI (Kg/m^2)	25.62 ± 0.55	44.66 ± 4.64	0.017*
FPG (mg/100 ml)	90.75 ± 1.75	95.62 ± 7.25	0.654
Insulin (μUI/ml)	16.85 ± 3.28	24.11 ± 2.99	0.163
HbA1c (%)	4.70 ± 0.43	5.46 ± 0.28	0.161
Total-C (mg/100 ml)	198.00 ± 3.67	209.00 ± 15.74	0.640
HDL-C (mg/100 ml)	52.25 ± 4.55	50.87 ± 2.52	0.770
Triglycerides (mg/100 ml)	129.00 ± 13.15	159.37 ± 19.17	0.322
Fibrinogen (mg/100 ml)	364.25 ± 44.95	449.75 ± 34.97	0.177
CRP (mg/100 ml)	0.37 ± 0.11	1.23 ± 0.19	0.012*

Values are expressed as the mean ± SEM

BMI body mass index, *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *Total-C* total cholesterol, *HDL-C* high density lipoprotein cholesterol, *CRP* C-reactive protein

*$P < 0.05$ of obese vs. non-obese individuals

Results

Clinical and anthropometric characteristics of the subjects

The characteristics of the patients are shown in Table 1. There was a significant difference in BMI between the two groups. Non-obese donors showed normal levels of the blood parameters evaluated. Differently, the obese patients had a mild alteration of glucose metabolism, but no diabetic subjects were involved in the study, as evidenced from the plasma levels of HbA1c. A mild dyslipidemia was also observed in obese, together with a significant increase of CRP blood values, compared to non-obese individuals.

Visceral ASC of non-obese subjects express high basal mRNA levels of SIRT1–6

We analyzed the basal expression of the seven SIRTs in both subcutaneous and visceral ASC isolated from non-obese and obese subjects. The evaluation and comparison of the ASC subpopulations from non-obese donors (nS-ASC vs. nV-ASC) showed that visceral ASC expressed significantly higher levels of SIRT1–6 compared to subcutaneous ASC (Fig. 1a). Differently, the ASC isolated from obese patients (obS-ASC vs. obV-ASC) did not reveal any significant differences in SIRTs' expression, although the visceral adipose-derived stem cells of obese patients (obV-ASC) showed a trend to lower expression of SIRT1-6 (Fig. 1b). SIRT7 had comparable basal expression in ASC of the two different AT sites, both in non-obese and obese subjects.

SIRT1–6 expression levels are markedly reduced in the obV-ASC

The above findings clearly indicate that obesity influences the expression of SIRTs in ASC. Further analysis of the data, performed to compare the SIRTs' expression in obese vs. non-obese subjects, highlighted indeed that the levels of SIRT1-6 were substantially lower in the obV-ASC than in the nV-ASC (Fig. 1d). Differently, the nS and obS-ASC had comparable levels of SIRT1–6, although the obS-ASC presented a non-significant trend to higher expression (Fig. 1c). Irrespective of the metabolic status of the donor, SIRT7 showed similar expression in both types of ASC.

SIRTs' mRNA expression correlates negatively with BMI and CRP and positively with PPARδ in visceral ASC

Possible correlations among the mRNA expression of the seven SIRTs and clinical parameters listed in Table 1 were evaluated on the whole cohort of individuals. The analysis unveiled significant inverse correlations between SIRT1–6 mRNA expression and BMI or CRP in visceral ASC (Table 2), whereas no association was evident in subcutaneous ASC (data not shown). As reported in our previous paper [22], the basal mRNA levels of PPARδ and PPARγ were analyzed in the same four ASC subpopulations in which SIRTs' expression was evaluated, showing that the level of PPARδ was substantially lower in the obV-ASC than in the nV-ASC. Table 3 shows significant positive correlations among the mRNA expression of SIRT1–6 and PPARδ in visceral ASC, whereas no correlation was found among SIRTs and PPARγ.

Hypoxia induces the SIRTs' mRNA expression only in the obV-ASC

As previously shown [22], we evaluated the possible contribution of the four ASC subpopulations to obesity-associated inflammation, showing that the mRNA levels of the inflammation-related genes NF-kB and NF-kB transcriptional targets were up-regulated in the obV-ASC upon 8 h of hypoxia exposure. In the present study, we tested whether SIRTs could also represent a possible target for hypoxic adaptation in ASC. Figure 2a shows that 8 h of oxygen deprivation did not induce significant changes of the SIRTs' expression in the two types of subcutaneous ASC compared to the basal value (indicated by a horizontal line in the graph). On the contrary, the subpopulations of visceral ASC displayed a significant modulation of several SIRT genes (Fig. 2b). In particular, all of the SIRTs were up-regulated in the obV-ASC, whereas SIRT5 and 6 were down-regulated and no significant change was observed for

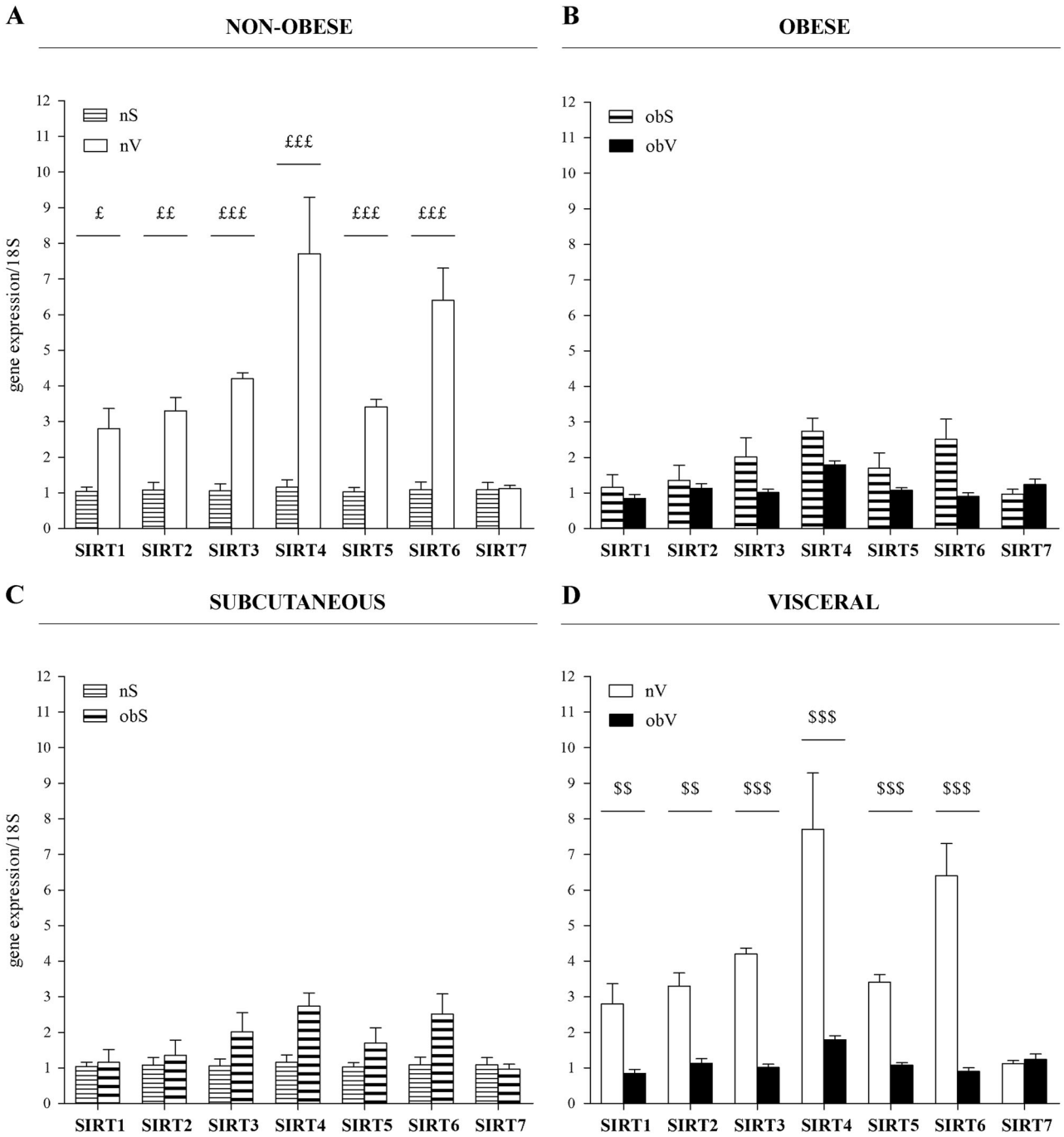

Fig. 1 Comparison between the constitutive expression level of each SIRT in subcutaneous vs. visceral ASC and in obese vs. non-obese individuals. Q-PCR showing mRNA expression of the seven SIRTs in the four subpopulations of ASC. The comparison between the expression level of each gene in the subcutaneous and visceral ASC of **a** the non-obese (nS- vs. nV-ASC) and **b** obese (obS- vs. obV-ASC) individuals is shown. The same data were further analyzed to compare the SIRTs' expression in **c** the two types of subcutaneous ASC (nS- vs. obS-ASC) and **d** visceral ASC (nV- vs. obV-ASC) of non-obese and obese subjects. The amount of each SIRT gene, normalized to the endogenous reference gene 18S and relative to the average of the four nS-ASC samples in basal conditions chosen as calibrator, was determined by the $2^{-\Delta\Delta Ct}$ method. The data are expressed as the mean ± SEM. $^{£}P < 0.05$, $^{££}P < 0.01$ and $^{£££}P < 0.001$ of nS-ASC vs. nV-ASC and $^{\$\$}P < 0.01$ and $^{\$\$\$}P < 0.001$ of nV-ASC vs. obV-ASC

SIRT1, 2, 3, 4 and 7 in the nV-ASC. However, after 8 h of oxygen deprivation, the levels of SIRT1–6 in the obV-ASC were significantly lower compared to those observed in the nV-ASC (Fig. 3).

Discussion

At present, there is no information regarding the expression of the complete panel of SIRTs in the ASC of the human fat

Table 2 Correlations among constitutive mRNA expression of SIRTs and BMI or CRP in visceral ASC

	BMI		CRP	
	r	*P*	*r*	*P*
SIRT1	−0.595	0.041*	−0.677	0.016*
SIRT2	−0.564	0.050*	−0.589	0.044*
SIRT3	−0.725	0.008**	−0.705	0.010**
SIRT4	−0.683	0.014*	−0.646	0.023*
SIRT5	−0.553	0.062	−0.790	0.002**
SIRT6	−0.725	0.008**	−0.660	0.020*
SIRT7	0.119	0.712	−0.277	0.383

Values are expressed as the mean ± SEM

*$P < 0.05$ and **$P < 0.01$ of BMI or CRP relative to the mRNA levels of each SIRT

Table 3 Correlations among basal mRNA expression of SIRTs and PPARδ or PPARγ in visceral ASC

	PPARδ		PPARγ	
	r	*P*	*r*	*P*
SIRT1	0.812	0.011*	−0.067	0.880
SIRT2	0.803	0.009**	−0.217	0.575
SIRT3	0.728	0.031*	0.067	0.880
SIRT4	0.778	0.017*	−0.050	0.912
SIRT5	0.770	0.021*	0.083	0.843
SIRT6	0.904	0.002**	−0.233	0.552
SIRT7	−0.075	0.843	−0.100	0.810

Values are expressed as the mean ± SEM.

*$P < 0.05$ and **$P < 0.01$ of the mRNA levels of PPARδ relative to those of each SIRT

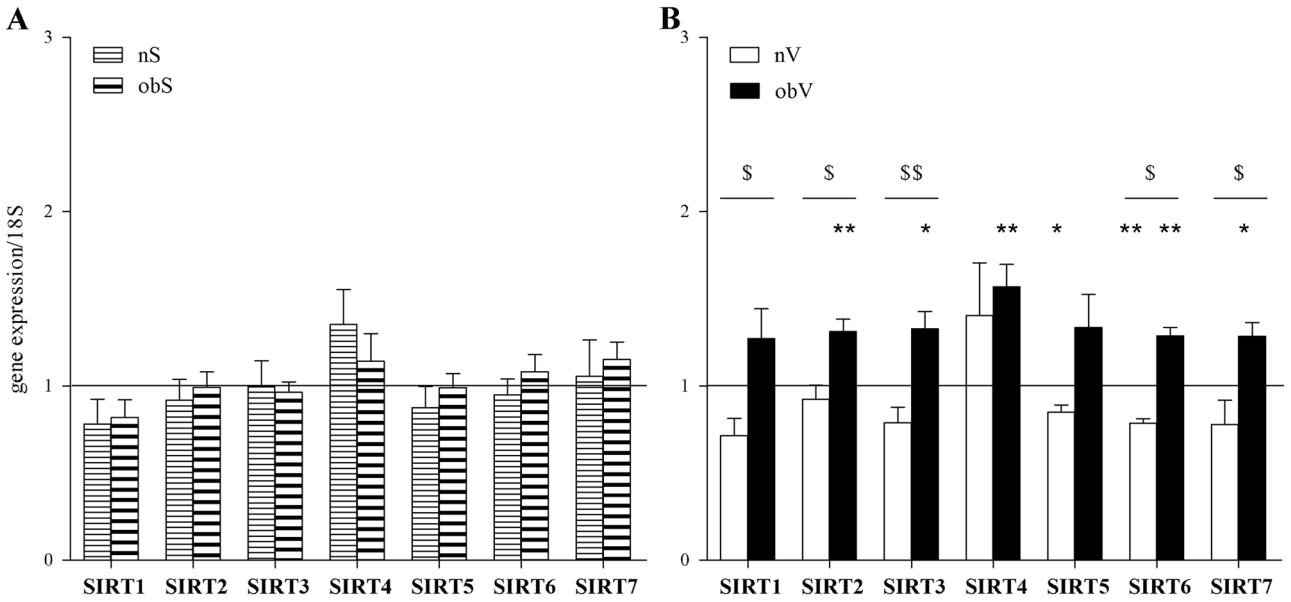

Fig. 2 Low oxygen determines up-regulation of the mRNA expression of SIRT1-7 compared to the basal levels in the obV-ASC. Q-PCR showing the changes in the mRNA levels of SIRTs in **a** the nS- and obS-ASC and **b** the nV- and obV-ASC after 8 h of oxygen deprivation. The amount of each SIRT gene, normalized to the endogenous control 18S and relative to the average of the four nS-ASC samples in basal conditions chosen as calibrator, was determined by the $2^{-\Delta\Delta Ct}$ method. All the values are expressed as the mean ± SEM. *$P < 0.05$ and **$P < 0.01$ of the effect of hypoxia relative to each untreated control, to which a value equal to 1 was arbitrarily assigned (horizontal lines in the graphs). $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ of nV-ASC vs. obV-ASC

depots. To address this issue, we investigated the mRNA expression levels of SIRTs in ASC isolated from SAT and VAT of non-obese and obese subjects. Our data showed very high levels of SIRT1–6 in the nV-ASC compared to the nS-ASC which could be related to the different metabolic activities of the two fat depots. It is consistent that the SIRTs' expression is higher in the nV-ASC, as they control pleiotropic metabolic pathways that increase the overall health of organisms [3–14]. Indeed, while SAT is deputed to store the excess of energy intake, such as free fatty acids (FFA) and glycerol that are deposited in the form of triglycerides, VAT has a larger effect on metabolism. VAT has a greater number of large adipocytes, which are more sensitive to lipolysis and insulin-resistant, thus contributing to the plasma FFA levels and glucose uptake. Moreover, it is more infiltrated with inflammatory cells and more capable of generating inflammatory markers, whereas has less preadipocyte differentiation capacity than SAT. Consequently, VAT accumulation is

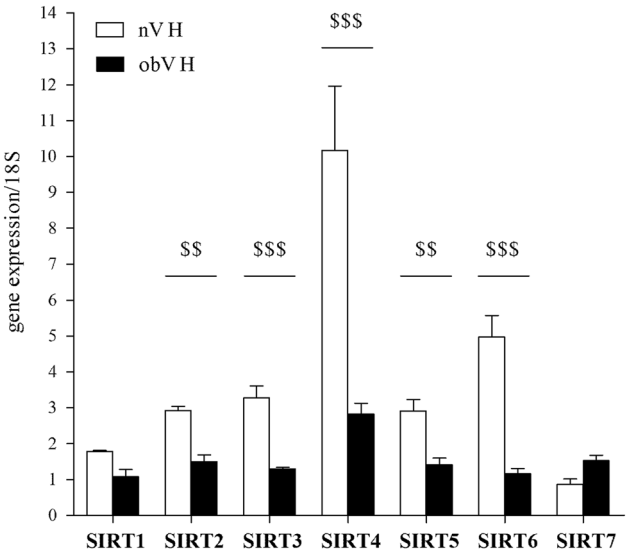

Fig. 3 Levels of SIRTs in the nV- and obV-ASC after hypoxia exposure. Q-PCR showing the mRNA expression of SIRTs in the nV- and obV-ASC after 8 h of hypoxia exposure (nV H and obV H, respectively). The amount of each SIRT gene, normalized to the endogenous control 18S and relative to the average of the four nS-ASC samples in basal conditions chosen as calibrator, was determined by the $2^{-\Delta\Delta Ct}$ method. The data are expressed as the mean ± SEM. $^{\$\$}P < 0.01$ and $^{\$\$\$}P < 0.001$ of nV-ASC vs. obV-ASC

associated with increased risk for diabetes, cardiovascular disease, hypertension and stroke [23].

The SIRTs' activity is regulated by their ability to act as energy sensors [1]. In fact, a low-energy status that increases cellular levels of NAD^+, such as fasting or calorie restriction, can activate SIRTs, whereas a high-energy status that decreases NAD^+ levels, including high-fat diet, reduces SIRT activity [24]. Consistently, several reports showed that SIRT1 expression is higher in lean compared to obese subjects [8, 9] and that transgenic mice over-expressing SIRT1 or mice treated with SIRT1 activators were protected against diet-induced obesity [25–27]. Conversely, AT-deletion of SIRT1 in mice fed with low-fat diet induced similar changes in gene expression than high-fat diet in wt mice, which showed reduced levels of SIRT1 mRNA and protein [28]. SIRT1 was reduced also in VAT and plasma of obese subjects with severe liver steatosis [29, 30], and negatively correlated with visceral fat areas [31]. Other studies showed that under calorie restriction SIRT1, 2 and 3 levels in AT increased, as well as SIRT1 in plasma [12, 32–34], whereas decrease of SIRT2 in VAT and SIRT4 in plasma was observed in obese [35, 36]. In agreement with all these findings, our results showed a strong reduction of the SIRTs' expression in the obV-ASC compared to the nV-ASC. Moreover, the observation that only in visceral ASC the mRNA levels of SIRT1–6 inversely correlated with BMI suggests a close link among SIRTs' expression and body fat amount and distribution. Accordingly, the inverse correlation between the plasma CRP levels of all of the individuals analyzed and the SIRTs' expression in the respective visceral ASC suggests that the protective effects of SIRTs concern also the obesity-associated inflammation, which is a peculiar feature of visceral obesity. Several reports showed that SIRT1, 2 and 6 exert anti-inflammatory effects through the inhibition of NF-kB signaling mediated by their deacetylase activity [6, 37–40]. In contrast, other studies described that SIRT1, 2 and 6 may be positive regulators of inflammatory processes [41–44]. It was suggested that NF-kB up-regulates SIRT1 at transcriptional level through the binding to a consensus NF-kB binding site on the promoter of SIRT1, also under inflammatory conditions [45, 46]. Interestingly, in addition to SIRT1, also SIRT2, 4 and 7 have potential NF-kB binding sites on their promoters (based on SABiosciences' proprietary DECODE database). In a recent study, performed on the same four ASC subpopulations used here, we analyzed the effects of hypoxia on the NF-kB pathway, highlighting that the obV-ASC exhibited hyper-responsiveness to hypoxia, which induced strong NF-kB activation and NF-kB target genes' expression, maximal after 2 and 8 h of oxygen deprivation, respectively [22]. In the present work, we evaluated whether SIRT genes could play a role in the NF-kB-mediated increase in inflammatory response. Actually, the previously observed NF-kB activation is consistent with the cell-specific up-regulation of the mRNA levels of all of the SIRTs observed after 8 h of hypoxia exposure solely in the obV-ASC, that we report here. Therefore, overall, our results support a transcriptional action of NF-kB on the SIRT genes in the obV-ASC. In agreement with Zhang et al., who suggested that SIRT1 up-regulation by NF-kB could be a ubiquitous auto-protective response of the cells to inflammatory events [45], we hypothesize that a similar regulatory mechanism may also exist in the obV-ASC. Indeed, the hypoxia-induced SIRTs' increase may result in a negative feedback loop controlling NF-kB signaling through the SIRTs' deacetylase activity to attenuate the deregulated inflammatory response of the obV-ASC. This notion is supported by the observation that the other three ASC subpopulations, that did not show NF-kB activation after 2 h of hypoxia exposure [22], do not exhibit even the enhancement of the SIRTs' expression. However, it should be noted that despite acute hypoxia increases the SIRTs' expression in the obV-ASC, the levels remained significantly lower than those of the nV-ASC. Consistently, the findings that the obV-ASC also constitutively expressed significantly lower levels of SIRT genes when compared to the nV-ASC, clearly indicate that in the obV-ASC, which underwent chronic exposition to an altered and hypoxia-remodeled AT microenvironment, the above proposed

auto-protective mechanism against inflammatory events is prevented.

In AT, PPARγ plays known functions such as adipocyte differentiation and up-regulation of genes involved in lipogenesis and triglyceride storage [47]. Consistently, we observed an inverse trend between PPARγ and SIRTs' expression. However, several effects of PPARδ in AT, opposite to those of PPARγ, have been also elucidated. Interestingly, like SIRTs, PPARδ is able to protect against obesity [15, 16]. In agreement with these findings, our results showed the existence of a positive relationship between PPARδ and SIRTs in visceral ASC suggesting that the protective effects of SIRTs may involve PPARδ. Accordingly, it has been recently shown that PPARδ up-regulates SIRT1 expression at the transcriptional level [48, 49]. These findings clearly show another possible regulator of SIRTs in AT.

Although, in our study, the assessment of SIRTs' expression was performed only at mRNA level, the results have high reproducibility and strong statistical significance.

In conclusion, this study for the first time analyzes the expression of the seven SIRTs in the ASC of both subcutaneous and visceral human fat depots of non-obese and obese subjects. The results highlight the influence of obesity on the SIRTs' expression, a possible mechanism through which SIRTs are involved in obesity-associated inflammation, as well as the interplay with PPARδ. Importantly, these findings also indicate interesting novel pathophysiological frameworks for SIRTs to explore in ASC.

Acknowledgments The authors thank Giuseppe Coroniti for his help.

Funding This study was funded by the Fondazione Roma and the Italian Ministry of Health (Ricerca Finalizzata RF-2011-02347907).

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. S. Imai, C.M. Armstrong, M. Kaeberlein, L. Guarente, Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature **403**, 795–800 (2000)
2. M.C. Haigis, D.A. Sinclair, Mammalian sirtuins: biological insights and disease relevance. Annu. Rev. Pathol. **5**, 253–295 (2010)
3. F. Picard, M. Kurtev, N. Chung, A. Topark-Ngarm, T. Senawong, R. Machado De Oliveira, M. Leid, M.W. McBurney, L. Guarente, Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature **429**, 771–776 (2004)
4. L. Qiang, L. Wang, N. Kon, W. Zhao, S. Lee, Y. Zhang, M. Rosenbaum, Y. Zhao, W. Gu, S.R. Farmer, D. Accili, Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Pparγ. Cell **150**, 620–632 (2012)
5. P. Chakrabarti, T. English, S. Karki, L. Qiang, R. Tao, J. Kim, Z. Luo, S.R. Farmer, K.V. Kandror, SIRT1 controls lipolysis in adipocytes via FOXO1-mediated expression of ATGL. J. Lipid. Res. **52**, 1693–1701 (2011)
6. T. Yoshizaki, J.C. Milne, T. Imamura, S. Schenk, N. Sonoda, J.L. Babendure, J.C. Lu, J.J. Smith, M.R. Jirousek, J.M. Olefsky, SIRT1 exerts anti-inflammatory effects and improves insulin sensitivity in adipocytes. Mol. Cell Biol. **29**, 1363–1374 (2009)
7. L. Qiao, J. Shao, SIRT1 regulates adiponectin gene expression through Foxo1-C/enhancer-binding protein alpha transcriptional complex. J. Biol. Chem. **281**, 39915–39924 (2006)
8. Y.S. Song, S.K. Lee, Y.J. Jang, H.S. Park, J.H. Kim, Y.J. Lee, Y. S. Heo, Association between low SIRT1 expression in visceral and subcutaneous adipose tissues and metabolic abnormalities in women with obesity and type 2 diabetes. Diabetes Res. Clin. Pract. **101**, 341–348 (2013)
9. S.B. Pedersen, J. Ølholm, S.K. Paulsen, M.F. Bennetzen, B. Richelsen, Low Sirt1 expression, which is upregulated by fasting, in human adipose tissue from obese women. Int. J. Obes. (Lond.) **32**, 1250–1255 (2008)
10. E. Jing, S. Gesta, C.R. Kahn, SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. Cell. Metab. **6**, 105–114 (2007)
11. F. Wang, Q. Tong, SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing FOXO1's repressive interaction with PPARgamma. Mol. Biol. Cell **20**, 801–808 (2009)
12. T. Shi, F. Wang, E. Stieren, Q. Tong, SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. J. Biol. Chem. **280**, 13560–13567 (2005)
13. 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**, 686–698 (2013)
14. Y. Kanfi, V. Peshti, R. Gil, S. Naiman, L. Nahum, E. Levin, N. Kronfeld-Schor, H.Y. Cohen, SIRT6 protects against pathological damage caused by diet-induced obesity. Aging Cell **9**, 162–173 (2010)
15. Y.X. Wang, C.H. Lee, S. Tiep, R.T. Yu, J. Ham, H. Kang, R.M. Evans, Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell **113**, 159–170 (2003)
16. T. Tanaka, J. Yamamoto, S. Iwasaki, H. Asaba, H. Hamura, Y. Ikeda, M. Watanabe, K. Magoori, R.X. Ioka, K. Tachibana, Y. Watanabe, Y. Uchiyama, K. Sumi, H. Iguchi, S. Ito, T. Doi, T. Hamakubo, M. Naito, J. Auwerx, M. Yanagisawa, T. Kodama, J. Sakai, Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. Proc. Natl. Acad. Sci. U S A **100**, 15924–15929 (2003)
17. P. Trayhurn, Hypoxia and adipose tissue function and dysfunction in obesity. Physiol. Rev. **93**, 1–21 (2013)

18. M.P. Gillum, M.E. Kotas, D.M. Erion, R. Kursawe, P. Chatterjee, K.T. Nead, E.S. Muise, J.J. Hsiao, D.W. Frederick, S. Yonemitsu, A.S. Banks, L. Qiang, S. Bhanot, J.M. Olefsky, D.D. Sears, S. Caprio, G.I. Shulman, SirT1 regulates adipose tissue inflammation. Diabetes **60**, 3235–3245 (2011)
19. T. Yoshizaki, S. Schenk, T. Imamura, J.L. Babendure, N. Sonoda, E.J. Bae, D.Y. Oh, M. Lu, J.C. Milne, C. Westphal, G. Bandyopadhyay, J.M. Olefsky, SIRT1 inhibits inflammatory pathways in macrophages and modulates insulin sensitivity. Am. J. Physiol. Endocrinol. Metab. **298**, E419–E428 (2010)
20. P.A. Zuk, M. Zhu, P. Ashjian, D.A. De Ugarte, J.I. Huang, H. Mizuno, Z.C. Alfonso, J.K. Fraser, P. Benhaim, M.H. Hedrick, Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell **13**, 4279–4295 (2002)
21. L. De Girolamo, D. Stanco, L. Salvatori, G. Coroniti, E. Arrigoni, G. Silecchia, M.A. Russo, S. Niada, E. Petrangeli, A.T. Brini, Stemness and osteogenic and adipogenic potential are differently impaired in subcutaneous and visceral adipose derived stem cells (ASCs) isolated from obese donors. Int. J. Immunopathol. Pharmacol. **26**, 11–21 (2013)
22. E. Petrangeli, G. Coroniti, A.T. Brini, L. de Girolamo, D. Stanco, S. Niada, G. Silecchia, E. Morgante, C. Lubrano, M.A. Russo, L. Salvatori, Hypoxia promotes the inflammatory response and stemness features in visceral fat stem cells from obese subjects. J. Cell. Physiol. **231**, 668–679 (2016)
23. M.M. Ibrahim, Subcutaneous and visceral adipose tissue: structural and functional differences. Obes. Rev. **11**, 11–18 (2010)
24. A. Chalkiadaki, L. Guarente, Sirtuins mediate mammalian metabolic responses to nutrient availability. Nat. Rev. Endocrinol. **8**, 287–296 (2012)
25. M. Lagouge, C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver, J. Auwerx, Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell **127**, 1109–1122 (2006)
26. J.A. Baur, K.J. Pearson, N.L. Price, H.A. Jamieson, C. Lerin, A. Kalra, V.V. Prabhu, J.S. Allard, G. Lopez-Lluch, K. Lewis, P.J. Pistell, S. Poosala, K.G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K.W. Fishbein, R.G. Spencer, E.G. Lakatta, D. Le Couteur, R.J. Shaw, P. Navas, P. Puigserver, D.K. Ingram, R. de Cabo, D.A. Sinclair, Resveratrol improves health and survival of mice on a high-calorie diet. Nature **444**, 337–342 (2006)
27. L. Bordone, D. Cohen, A. Robinson, M.C. Motta, E. van Veen, A. Czopik, A.D. Steele, H. Crowe, S. Marmor, J. Luo, W. Gu, L. Guarente, SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell **6**, 759–767 (2007)
28. A. Chalkiadaki, L. Guarente, High-fat diet triggers inflammation-induced cleavage of SIRT1 in adipose tissue to promote metabolic dysfunction. Cell Metab. **16**, 180–188 (2012)
29. Co.S. Costa, T.O. Hammes, F. Rohden, R. Margis, J.W. Bortolotto, A.V. Padoin, C.C. Mottin, R.M. Guaragna, SIRT1 transcription is decreased in visceral adipose tissue of morbidly obese patients with severe hepatic steatosis. Obes. Surg. **20**, 633–639 (2010)
30. S. Mariani, D. Fiore, S. Basciani, A. Persichetti, S. Contini, C. Lubrano, L. Salvatori, A. Lenzi, L. Gnessi, Plasma levels of SIRT1 associate with non-alcoholic fatty liver disease in obese patients. Endocrine **49**, 711–716 (2015)
31. H. Lee, S.H. Chu, J.Y. Park, H.K. Park, J.A. Im, J.W. Lee, Visceral adiposity is associated with SIRT1 expression in peripheral blood mononuclear cells: a pilot study. Endocr. J. **60**, 1269–1273 (2013)
32. H.Y. Cohen, C. Miller, K.J. Bitterman, N.R. Wall, B. Hekking, B. Kessler, K.T. Howitz, M. Gorospe, R. de Cabo, D.A. Sinclair, Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science **305**, 390–392 (2004)
33. F. Wang, M. Nguyen, F.X. Qin, Q. Tong, SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. Aging Cell **6**, 505–514 (2007)
34. S. Mariani, D. Fiore, A. Persichetti, S. Basciani, C. Lubrano, E. Poggiogalle, A. Genco, L.M. Donini, L. Gnessi, Circulating SIRT1 Increases after intragastric balloon fat loss in obese patients. Obes. Surg. **26**, 1215–1220 (2016)
35. J. Krishnan, C. Danzer, T. Simka, J. Ukropec, K.M. Walter, S. Kumpf, P. Mirtschink, B. Ukropcova, D. Gasperikova, T. Pedrazzini, W. Krek, Dietary obesity-associated Hif1α activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. Genes. Dev. **26**, 259–270 (2012)
36. 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. Oxid. Med. Cell. Longev. **2014**, 920676 (2014)
37. K.M. Rothgiesser, S. Erener, S. Waibel, B. Lüscher, M.O. Hottiger, SIRT2 regulates NF-κB dependent gene expression through deacetylation of p65 Lys310. J. Cell Sci. **123**, 4251–4258 (2010)
38. T.L. Kawahara, E. Michishita, A.S. Adler, M. Damian, E. Berber, M. Lin, R.A. McCord, K.C. Ongaigui, L.D. Boxer, H.Y. Chang, K.F. Chua, SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. Cell **136**, 62–74 (2009)
39. F. Yeung, J.E. Hoberg, C.S. Ramsey, M.D. Keller, D.R. Jones, R. A. Frye, M.W. Mayo, Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J. **23**, 2369–2380 (2004)
40. S. Rajendrasozhan, S.R. Yang, V.L. Kinnula, I. Rahman, SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. **177**, 861–870 (2008)
41. C.A. Fernandes, L. Fievez, A.M. Neyrinck, N.M. Delzenne, F. Bureau, R. Vanbever, Sirtuin inhibition attenuates the production of inflammatory cytokines in lipopolysaccharide-stimulated macrophages. Biochem. Biophys. Res. Commun. **420**, 857–861 (2012)
42. A. Legutko, T. Marichal, L. Fiévez, D. Bedoret, A. Mayer, H. de Vries, L. Klotz, P.V. Drion, C. Heirman, D. Cataldo, R. Louis, K. Thielemans, F. Andris, O. Leo, P. Lekeux, C.J. Desmet, F. Bureau, Sirtuin 1 promotes Th2 responses and airway allergy by repressing peroxisome proliferator-activated receptor-γ activity in dendritic cells. J. Immunol. **187**, 4517–4529 (2011)
43. F. Van Gool, M. Gallí, C. Gueydan, V. Kruys, P.P. Prevot, A. Bedalov, R. Mostoslavsky, F.W. Alt, T. De Smedt, O. Leo, Intracellular NAD levels regulate tumor necrosis factor protein synthesis in a sirtuin-dependent manner. Nat. Med. **15**, 206–210 (2009)
44. S. Bruzzone, F. Fruscione, S. Morando, T. Ferrando, A. Poggi, A. Garuti, A. D'Urso, M. Selmo, F. Benvenuto, M. Cea, G. Zoppoli, E. Moran, D. Soncini, A. Ballestrero, B. Sordat, F. Patrone, R. Mostoslavsky, A. Uccelli, A. Nencioni, Catastrophic NAD+ depletion in activated T lymphocytes through Nampt inhibition reduces demyelination and disability in EAE. PLoS One **4**, e7897 (2009)
45. H.N. Zhang, L. Li, P. Gao, H.Z. Chen, R. Zhang, Y.S. Wei, D.P. Liu, C.C. Liang, Involvement of the p65/RelA subunit of NF-kappaB in TNF-alpha-induced SIRT1 expression in vascular

smooth muscle cells. Biochem. Biophys. Res. Commun. **397**, 569–575 (2010)
46. J. Katto, N. Engel, W. Abbas, G. Herbein, U. Mahlknecht, Transcription factor NFκB regulates the expression of the histone deacetylase SIRT1. Clin. Epigenetics **5**, 11 (2013)
47. A.M. Sharma, B. Staels, Review: peroxisome proliferator-activated receptor gamma and adipose tissue--understanding obesity-related changes in regulation of lipid and glucose metabolism. J. Clin. Endocrinol. Metab. **92**, 386–395 (2007)
48. M. Okazaki, Y. Iwasaki, M. Nishiyama, T. Taguchi, M. Tsugita, S. Nakayama, M. Kambayashi, K. Hashimoto, Y. Terada, PPARbeta/delta regulates the human SIRT1 gene transcription via Sp1. Endocr. J. **57**, 403–413 (2010)
49. J.S. Hwang, W.J. Lee, E.S. Kang, S.A. Ham, T. Yoo, K.S. Paek, D.S. Lim, J.T. Do, H.G. Seo, Ligand-activated peroxisome proliferator-activated receptor-δ and -γ inhibit lipopolysaccharide-primed release of high mobility group box 1 through upregulation of SIRT1. Cell. Death. Dis. **5**, e1432 (2014)