Weight Loss Is Associated With Increased NAD$^+$/SIRT1 Expression But Reduced PARP Activity in White Adipose Tissue

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Context: Sirtuins (SIRTs) and poly(ADP-ribose) polymerases (PARPs) are 2 important nicotinamide adenine dinucleotide (NAD)$^+$-dependent enzyme families with opposing metabolic effects. Energy shortage increases NAD$^+$ biosynthesis and SIRT activity but reduces PARP activity in animals. Effects of energy balance on these pathways in humans are unknown.

Objective: We compared NAD$^+$/SIRT pathway expressions and PARP activities in sc adipose tissue (SAT) between lean and obese subjects and investigated their change in the obese subjects during a 12-month weight loss.

Design, Setting and Participants: SAT biopsies were obtained from 19 clinically healthy obese subjects (mean $\pm$ SE body mass index, 34.6 $\pm$ 2.7 kg/m^2) during a weight-loss intervention (0, 5, and 12 mo) and from 19 lean reference subjects (body mass index, 22.7 $\pm$ 1.1 kg/m^2) at baseline.

Main Outcome Measures: SAT mRNA expressions of *SIRT*s 1–7 and the rate-limiting gene in NAD$^+$ biosynthesis, nicotinamide phosphoribosyltransferase (*NAMPT*) were measured by Affymetrix, and total PARP activity by ELISA kit.

Results: *SIRT1*, *SIRT3*, *SIRT7*, and *NAMPT* expressions were significantly lower, whereas total PARP activity was increased in obese compared with lean subjects. *SIRT1* and *NAMPT* expressions increased in obese subjects between 0 and 5 months, after a mean weight loss of 11.7%. In subjects who continued to lose weight between 5 and 12 months, *SIRT1* expression increased progressively, whereas in subjects with weight regain, *SIRT1* reverted to baseline levels. PARP activity significantly decreased in all subjects upon weight loss.

Conclusions: Calorie restriction is an attractive strategy to improve the NAD$^+$/SIRT pathway and decrease PARPs in SAT in human obesity. (***J Clin Endocrinol Metab*** **101: 1263–1273, 2016)**

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Abbreviations: BAT, brown adipose tissue; BMI, body mass index; BP, blood pressure; CR, calorie restriction; HDL, high-density lipoprotein; HFD, high-fat diet; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAD, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; PARP, poly(ADP-ribose) polymerase; PET, positron emission tomography; SAT, subcutaneous adipose tissue; SIRT, sirtuin; VAT, visceral adipose tissue.

Obesity is one of today's most alarming public health problems worldwide, significantly increasing the risk of several chronic diseases, such as type 2 diabetes, metabolic syndrome, coronary heart disease, hypertension, stroke and cancers (1). The development of these diseases likely involves several factors, one of which is adipose tissue dysfunction, characterized by down-regulation of mitochondrial biogenesis, increased inflammation and oxidative stress (2–4). Mitochondrial function, metabolism and cellular stress response are controlled by a family of nicotinamide adenine dinucleotide $(NAD)^+$-dependent enzyme families, sirtuins (SIRTs) (SIRT1–SIRT7) and poly(ADP-ribose) polymerases (PARPs) (PARP1–PARP18), which use NAD^+ as their substrate (5). SIRTs are activated by an increase in NAD^+ availability via stimulation of the rate-limiting enzyme in NAD^+ biosynthesis pathway, nicotinamide phosphoribosyltransferase (NAMPT) during exercise and nutrient shortage (5). Upon activation, SIRTs deacylate or mono-ADP-ribosylate their target proteins to boost mitochondrial oxidative metabolism and protect from oxidative stress caused by reactive oxygen species (5, 6). PARPs are major consumers of NAD^+ in cells as they cleave NAD^+ to form poly(ADP-ribose) polymers on their target proteins. Several physiological situations such as aging, oxidative stress and DNA damage stimulate PARPs. Mouse studies have suggested that PARPs and SIRT1 compete for the same intracellular NAD^+ pool and PARP activity most likely has a significant impact on SIRT1 activity. For example, during a high-fat diet (HFD), PARPs are induced (6), whereas SIRT1 activity (7) and NAD^+ biosynthesis (8) are severely compromised. Therefore, a functional interaction most likely exists between SIRT1 and PARPs, but this interaction is still largely unexplored in humans.

Of the 7 SIRTs, SIRT1 is the most studied and known to have a prominent role in the control of metabolic health. *Sirt1* overexpression (9) or pharmacological activation of SIRT1 (10) enhance energy expenditure and reduce body fat gain. In addition, most findings support the notion that SIRT1 serve as an insulin sensitizer in mice (10), primates (11), and humans (12). Of the other SIRTs, SIRT3 (13), SIRT5 (14), SIRT6 (15), and SIRT7 (16) have also been suggested to facilitate lipid utilization and/or mitochondria function in mice. Also, PARPs have recently been recognized to have an important role in metabolism. Deletion of *Parp1* and *Parp2* genes (6, 17) and pharmacological PARP inhibition (18) mitigate HFD-induced obesity and increase insulin sensitivity.

A majority of the studies of SIRTs and PARPs have concentrated on nonadipose tissues, and data on their function in white sc adipose tissue (SAT) during changes in energy balance are scarce in humans. However, SIRT1 is an exception, as a number of studies have shown its expression to be down-regulated in obesity and up-regulated during calorie restriction (CR) in SAT as well as in other tissues both in rodents (5, 6) and in humans (19–21). Further, in morbidly obese patients undergoing gastric banding surgery, *SIRT3* and *SIRT6* expressions were up-regulated in SAT after 6 months (21). However, no data exist for long-term effects of weight changes on NAD^+/SIRT pathway in humans. Also, *SIRT1–SIRT7* expressions have not been compared between lean and obese subjects. The effect of obesity or CR on PARPs remains yet unexplored in humans, but fasting is known to lower PARP activity in muscle in mice (6).

To elucidate this unexplored field, we performed a comprehensive study on the 2 NAD^+-dependent enzyme families, SIRTs and PARPs, comparing their levels in SAT in lean and obese volunteers as well as in the obese subjects during a 12-month weight loss program. Similarly, we examined the SAT pathways for oxidative stress and inflammation, as they have close biological connections to mitochondria function, as well as with SIRTs and PARPs. Detailed clinical data enabled correlations to be made between metabolic parameters and the SAT expression and enzyme activities.

Materials and Methods

Study design

Nineteen clinically healthy obese volunteers (7 males, 12 females) with mean ± SE body mass index (BMI) of 34.6 ± 2.7 kg/m^2, aged 35.2 ± 1.8 years, participated in a 12-month weight loss program. Weight development and metabolic health were assessed at 0, 5, and 12 months. The examination protocol is described in Supplemental Figure 1. Nineteen sex- and age-matched healthy lean reference subjects (7 males, 12 females) with BMI 22.7 ± 0.2, aged 32.6 ± 0.9, were also examined at baseline in a similar manner as obese subjects excluding magnetic resonance imaging (MRI) and positron emission tomography (PET) scannings. The ethics committees of the hospital districts of Southwest Finland and Helsinki and Uusimaa approved the study protocol, and the study was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Weight-loss intervention

Detailed description of the weight loss protocol has been published previously (22). In brief, obese subjects participated in a 12-month intervention program, consisting of individual and group-based diet and exercise counseling. It started with a 6-week modified very-low-energy diet phase providing about 800–1000 kcal per day. The subjects were thereafter advised to continue a 500- to 1000-kcal restriction from baseline diet. To assess habitual dietary intake, the participants were instructed to record their food intake by keeping a detailed food diary for consecutive 3 days at 0, 5, and 12 months.

Clinical assessments

After a 12-hour overnight fast, weight and height were measured in underwear. We also measured waist circumference midway between the anterior superior iliac spine and the lower rib margin and body composition by dual-energy x-ray absorptiometry (GE Lunar Prodigy). Blood pressure (BP) was measured by a mercury sphygmomanometer as mean of 3 consecutive measurements.

The volumes of SAT and visceral adipose tissue (VAT) were measured using MRI, liver fat content using magnetic resonance spectroscopy (MRS) (23), and glucose uptake in SAT, VAT, and brown adipose tissue (BAT) using 2-deoxy-2-(^{18}F)fluoro-D-glucose-PET during cold exposure (24) at baseline and 5 months in obese subjects (Supplemental Figure 1). To restrict isotope exposure, the studies were not repeated at 12 months. In reference subjects, liver fat was measured using MRS as previously described (2, 25).

A 75-g oral glucose tolerance test with 4 time points (0, 30, 60, and 120 min) was performed after a 12-hour overnight fast, followed by measurements of plasma glucose (spectrophotometric hexokinase and glucose-6-phosphate dehydrogenase assay; Roche Diagnostics) and serum insulin (time-resolved immunofluorometric assay; PerkinElmer), and calculations of homeostatic model assessment (HOMA) insulin resistance and Matsuda insulin sensitivity indexes as in (2). Fasting plasma total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride concentrations were determined with enzymatic methods (Roche Diagnostics Hitachi, Hitachi Ltd). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald formula.

SAT analyses

Biopsies of periumbilical SAT were obtained under local anesthesia by a surgical technique and snap frozen in liquid nitrogen. A part of the biopsy was treated with collagenase to measure mean adipocyte diameter under a light microscope (26).

Gene expression studies in SAT

Total RNA was extracted from SAT using the protocol described earlier (3). Transcriptomics analyses (Affymetrix U133 Plus 2.0 array) were performed and validated as published previously (2). *SIRT1*–*SIRT7* mRNA expressions were picked from the transcriptomics data. Quantitative RT-PCR was used to validate some of the microarray data (please see Supplemental Methods for details).

Total PARP activity measurement

SAT biopsy specimens of 16 obese subjects and 11 lean reference subjects were available for the analysis of total PARP activity at baseline, whereas the effect of weight loss was studied in 13 SAT biopsies of obese subjects at baseline and 12 months due to limitations in the SAT material at all time points. Total PARP activity measurement was performed using a HT Colorimetric PARP/Apoptosis ELISA kit (catalog 4684-096-K; Trevigen, Trevigen, Inc) following the kit's instructions. Results were normalized using a DNA concentration measured with Qubit 2.0 Fluorometer (Life Technologies).

Statistical analyses

Results are expressed as mean ± SE for normally distributed variables and median (interquartile range) for skewed variables. Comparisons between reference group and obese subjects at baseline were made with *t* test for normally distributed variables and with Mann-Whitney *U* test for skewed variables. For further statistical analyses, natural logarithmic transformations were performed on variables with a skewed distribution (BAT glucose uptake, VAT, liver fat percentage, plasma triglycerides, HOMA index, Matsuda index). Comparisons between the 0-, 5-, and 12-month time points in obese subjects during weight loss were analyzed by repeated measures ANOVA with Wald test for post hoc comparisons. Continuous associations between variables were assessed via partial correlations adjusting for age and sex and visualized by partial regression plots adjusted for age and sex. The statistical power for comparisons in Figure 1 and Figure 3 below with subdivision of the participants into 2 groups were as follows: continuous weight loss from 0 to 12 months (n = 6) and weight regain from 5 to 12 months (n = 13) was limited. Using power calculations for *t* tests as conservative estimates for the power of post hoc Wald tests comparisons, the power to detect "large" effect sizes of Cohen's $d = 0.8$ was 0.33 for between group comparisons, 0.36 for within group comparisons in the continuous weight loss group and 0.75 for within group comparisons in the weight regainer group (0.80 power being the golden standard).

Results

Clinical and metabolic characteristics of study subjects

Baseline characteristics of the obese and lean reference subjects and changes in obese subjects at 5 and 12 months are shown in Table 1. All subjects were clinically healthy, but as expected, obese subjects had higher BMI, more body fat, larger waist circumference and adipocyte size, fasting glucose, insulin, and HOMA index, lower Matsuda index, higher BP and triglyceride, and lower HDL cholesterol levels than reference subjects. The reported daily energy intake did not differ significantly between the groups.

The effect of weight loss on clinical and metabolic characteristics

The total energy intake decreased between 0 and 5 months by an average of 35.2%, resulting in a mean weight loss of 11.6 ± 1.3 kg (−11.7%), with concomitant improvements in fasting glucose and insulin levels, HOMA index, Matsuda index, total cholesterol, LDL cholesterol, triglyceride levels and systolic BP (Table 1). Between 5 and 12 months, the mean total energy intake increased by 26.4%, paralleled by a slight mean weight regain (2.7 kg). The net weight loss from baseline to the end of the intervention was 9.1 kg (−9.0%). Overall, fasting glucose, LDL and HDL cholesterol, triglycerides, and systolic BP remained improved in the entire group at 12 months.

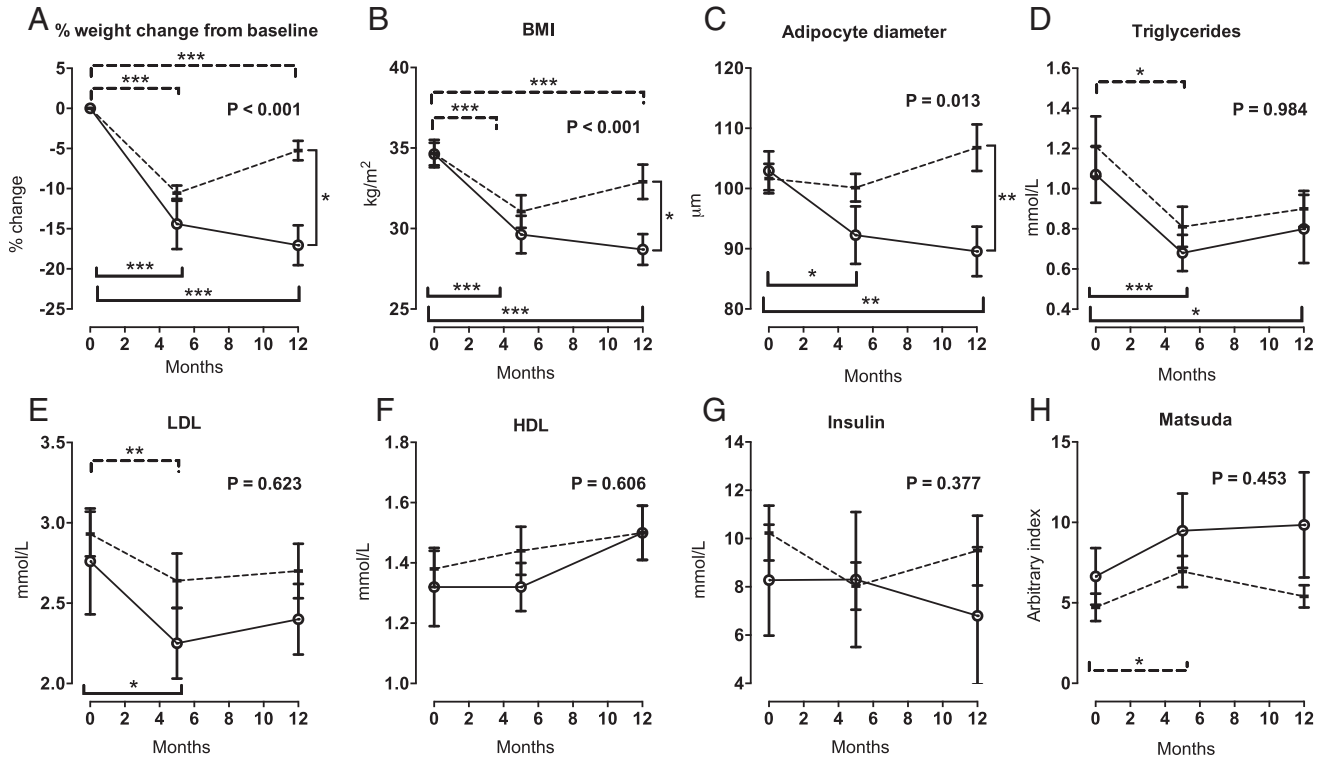

Figure 1. Selected clinical and metabolic characteristics of obese subjects before and during the long-term weight loss program in 2 separate groups: continuous weight losers (n = 6) (solid line) and weight regainers (n = 13) (dashed line). BMI (A), percentage weight loss (B), adipocyte diameter (C), triglycerides (D), LDL cholesterol (E), HDL cholesterol (F), fasting insulin levels (G), and Matsuda index (H). Data shown as mean ± SE. *, $P < .05$; **, $P < .01$; ***, $P < .001$; *P* values were calculated with repeated measures ANOVA using Wald tests as post hoc tests. *P* values in the figure correspond to global *P* values for the ANOVA.

Using MRI, MRS, and PET imaging, we determined the sizes and metabolic activity of fat depots during the most intensive period of weight loss between baseline and 5 months (Table 1). The amount of SAT and VAT as well as liver fat percentage showed a significant decline during weight loss. Cold-induced glucose uptake of BAT measured by 2-deoxy-2-(^{18}F)fluoro-D-glucose -PET, tended to increase by 34.1% after 5 months of intervention, but no changes were found in SAT, VAT, and liver glucose uptake in response to the weight loss.

Two groups emerge: continuous weight losers and weight regainers

After 5 months, 6 of the patients continued to lose weight, with a mean weight loss of 17.5 kg (−17.1%) at 12 months (Figure 1A). The other 13 patients (who either maintained their weight or started to regain after 5 mo) had a mean of 4.9 kg (−5.1%) total weight loss at 12 months (Figure 1A).

The emergence of 2 distinct groups allowed us to analyze the metabolic parameters separately during continuous weight loss and weight regain. In the patients with continuous weight loss, we observed significant decrease in weight (Figure 1A), BMI (Figure 1B), adipocyte size (Figure 1C), and triglycerides (Figure 1D) during the intervention between 0 and 5 months and up to 12 months. In the remaining subjects, the initial metabolic improvements were diminished or lost by 12 months.

The effect of obesity and weight loss on *SIRT* mRNA levels, total PARP activity and NAD$^+$ biosynthesis in SAT

Next, we investigated with Affymetrix microarray whether *SIRT*s and *NAMPT* mRNA levels in SAT differed between reference and obese subjects. SAT *SIRT1* (Figure 2A), *SIRT3* (Figure 2C), *SIRT7* (Figure 2E), and *NAMPT* (Figure 2F) mRNA levels were significantly higher in reference than in obese subjects at baseline, whereas *SIRT2* (Figure 2B) and *SIRT5* (Figure 2D) did not significantly differ between the groups. *SIRT4* and *SIRT6* were not detected by the microarray in SAT. *SIRT1* and *NAMPT* results were validated by quantitative RT-PCR (Supplemental Figures 2A and 3A, respectively). In contrast, total PARP activity was significantly higher in obese subjects when compared with reference subjects (Figure 2G) despite of unchanged *PARP1* and *PARP2* gene expression levels (Supplemental Figure 3, C and D).

In response to weight loss, *SIRT1* mRNA levels were significantly increased in SAT both at 5 and at 12 months (Figure 3A; validation by quantitative RT-PCR in Supple-

Table 1. Clinical and Metabolic Characteristics of Obese Subjects Before and After the Long-Term Weight Loss Program As Well As of Reference Subjects at Baseline

	Reference Subjects	Obese Subjects			*P* Values*			
Variables	0 Months n = 19	0 Months n = 19	5 Months n = 19	12 Months n = 19	Obese vs ref.	Obese 0–5 Months	Obese 5–12 Months	Obese 0–12 Months
Weight (kg)	67.4 (2.6)	99.0 (3.2)	87.4 (3.3)	90.1 (3.4)	.0001	<.0001	.0145	.0001
Height (cm)	171.5 (2.8)	168.7 (2.2)	NA	NA	.4431	NA	NA	NA
BMI (kg/m^2)	22.7 (0.3)	34.6 (0.6)	30.6 (0.8)	31.6 (3.9)	<.0001	<.0001	.0114	.0001
Body fat (%)	25.3 (1.9)	44.6 (1.6)	39.6 (2.0)	40.6 (9.3)	<.0001	<.0001	.1696	.0009
Waist (cm)	76.2 (1.5)	112.5 (2.3)	100.1 (2.6)	99.4 (11.8)	<.0001	<.0001	.4215	<.0001
Adipocyte diameter (μm)	75.4 (2.4)	102.1 (1.9)	97.8 (2.4)	100.4 (3.5)	<.0001	.1499	.1329	.6479
fP-glucose (mmol/L)	5.1 (0.1)	5.6 (0.10)	5.4 (0.1)	5.4 (0.7)	.0001	.0132	.6763	.0463
fS-insulin (mU/L)	3.3 (1.8–5.1)	8.1 (5.8–12.9)	7.3 (4.7–10.7)	6.8 (5.8–11.3)	<.0001	.1988	.6040	.2924
HOMA index	0.7 (0.4–1.3)	1.9 (1.5–3.3)	1.9 (1.0–2.5)	1.6 (1.3–3.2)	<.0001	.1347	.5091	.2442
Matsuda index	9.7 (6.6–17.2)	4.5 (2.7–6.2)	6.6 (4.6–11.0)	5.9 (4.7–8.0)	.0003	.0063	.4175	.2352
Total cholesterol (mmol/L)	4.5 (0.2)	4.6 (0.2)	4.2 (0.2)	4.4 (0.7)	.6482	.0037	.0612	.1277
LDL cholesterol (mmol/L)	2.8 (0.2)	2.9 (0.1)	2.5 (0.1)	2.6 (0.6)	.5729	.0059	.5623	.0134
HDL cholesterol (mmol/L)	1.7 (0.1)	1.4 (0.1)	1.4 (0.1)	1.5 (0.3)	.0186	.3785	.1043	.0267
Triglycerides (mmol/L)	0.7 (0.6–1.0)	0.9 (1.5–3.3)	0.7 (0.5–0.9)	0.8 (0.6–1.1)	.0055	.0011	.0704	.0138
Systolic BP (mm Hg)	119.8 (1.9)	135.1 (3.4)	117.8 (2.2)	125.1 (2.9)	.0004	<.0001	.0060	.0021
Diastolic BP (mm Hg)	74.7 (1.6)	83.8 (1.9)	81.3 (1.8)	82.1 (2.7)	.0008	.2687	.6924	.4067
Total energy intake (kcal/d)	2237.1 (108.8)	2457.9 (212.0)	1593.4 (109.9)	2013.0 (182.5)	.3530	.0005	.0401	.0901
SAT (kg)	NA	13.8 (0.8)	9.8 (0.9)	NA	NA	<.0001	NA	NA
Intraabdominal adipose tissue (kg)	NA	3.7 (0.6)	2.3 (0.4)	NA	NA	<.0001	NA	NA
Liver fat (%)	0.67 (0.08)	6.7 (1.1)	1.8 (0.5)	NA	<.0001	<.0001	NA	NA
SAT glucose uptake (μmol/kg^{-1} · min^{-1})	NA	0.9 (0.04)	0.8 (0.03)	NA	NA	.5062	NA	NA
Intraabdominal adipose tissue glucose uptake (μmol/kg^{-1} · min^{-1})	NA	2.2 (0.1)	2.1 (0.1)	NA	NA	.5370	NA	NA
BAT glucose uptake (μmol/kg^{-1} · min^{-1})	NA	4.4 (1.4)	5.9 (1.7)	NA	NA	.1581	NA	NA
Liver glucose uptake (μmol/kg^{-1} · min^{-1})	NA	2.2 (0.2)	2.7 (0.3)	NA	NA	.1934	NA	NA

NA, not analyzed. Data shown as mean (SE) and for skewed variables median (interquartile range). Comparisons between reference and obese subjects at baseline were made with unpaired *t* test for normally distributed and with Mann-Whitney *U* test for skewed variables. Comparisons between different time points in obese group were made with paired *t* tests for normally distributed and with Wilcoxon signed-rank test for skewed variables.

mental Figure 2, B and C). In addition, *SIRT3* mRNA levels decreased (Figure 3C), whereas *SIRT7* mRNA levels increased significantly between baseline and 12 months (Figure 3E). The mRNA levels of *SIRT2* (Figure 3B) and *SIRT5* did not change in response to weight loss (Figure 3D).

We then focused on the expression of *SIRT1* in the 2 subgroups: the continuous weight losers and the weight regainers. Interestingly, *SIRT1* mRNA levels were significantly higher at baseline among the subjects who kept losing weight until the end of intervention (Figure 3F). SAT *SIRT1* mRNA levels increased upon weight loss until 5 months both in the continuous weight losers and in the weight regainers, but the levels further increased only with continued weight loss while reverted back to the baseline at 12 months in the weight regainers (Figure 3F). Overall, *SIRT1* mRNA levels (Figure 3F) tended to follow inversely the trend of weight and BMI (Figure 1, A and B, respectively) during the weight-loss intervention.

According to the literature, fasting lowers PARP activity in rodents. Thus, we hypothesize that long-term weight loss could be associated with decreased PARP enzyme activity in SAT. Indeed, our results showed that the total PARP activity was significantly decreased (38.2%) in SAT after a 12-month long-term weight-loss intervention (Figure 3G). In addition, *PARP1* gene expression significantly reduced between 0 and 12 months, whereas expression of *PARP2* was unaffected upon weight loss (Supplemental Figure 3, E and F). Last, because both SIRT1 and PARP1 are NAD$^+$-dependent enzymes, we measured the mRNA levels of *NAMPT*, a key enzyme in NAD$^+$ biosynthesis. Consistent with the hypothesis that NAD$^+$ levels would be increased after weight loss, we observed that *NAMPT* was significantly up-regulated (by as much as 43.0%) between baseline and 5 months (Figure 3H). These results were further consolidated by quantitative RT-PCR results (Supplemental Figure 2, B and C).

The effect of obesity and weight loss on inflammation and oxidative stress

Because PARPs and SIRTs are connected with inflammation and oxidative stress, we investigated inflammation and oxidative stress-related gene expression pathways. Inflammation-related genes were up-regulated in the obese subjects compared with the reference group (Supplemen-

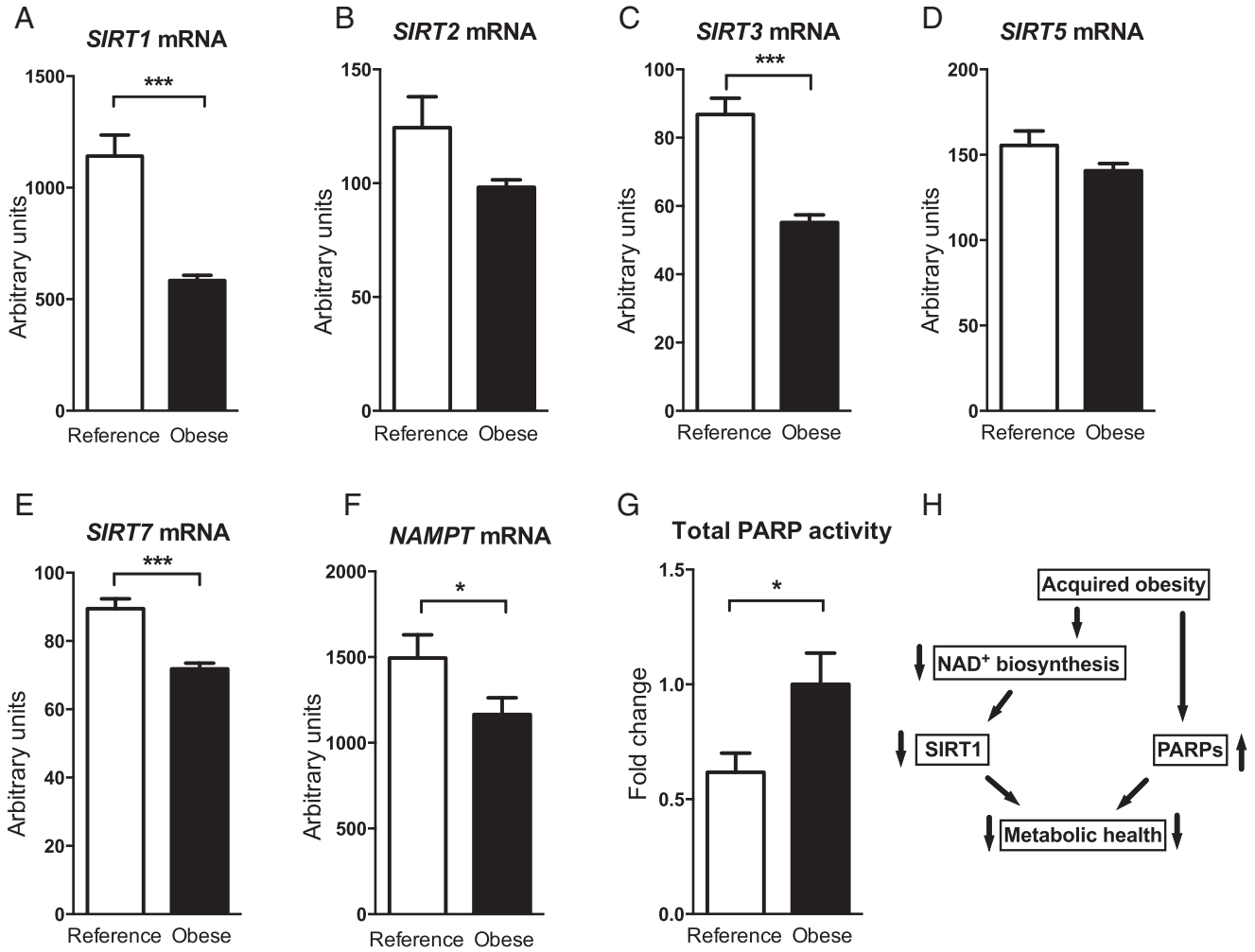

Figure 2. *SIRT*s and *NAMPT* mRNA levels in SAT at baseline (0 mo) between 2 study groups: reference group (white column) and obese subjects (black column). *SIRT1* (A), *SIRT2* (B), *SIRT3* (C), *SIRT5* (D), *SIRT7* (E), and *NAMPT* (F) mRNA levels. Arbitrary units denote Affymetrix mRNA values after normalization. Total PARP enzyme activity (n = 11 in reference and n = 16 in obese subjects) (G). Suggested effects of obesity on metabolic health via SIRT1 and PARPs (H). SIRT1 is deactivated via decreased NAD^+ availability due to suppressed NAD^+ biosynthesis and induced PARP activity. Poor dietary habits may activate PARPs, which promote metabolic impairment and inflammation. Data shown as mean ± SE. *, $P < .05$; **, $P < .01$; ***, $P < .001$. *P* values were calculated with unpaired *t* tests.

tal Figure 4A), whereas no significant differences were observed for oxidative stress-related genes (Supplemental Figure 4D). Surprisingly, no effect of weight loss was observed for inflammation (Supplemental Figure 4B), but oxidative stress-related genes were down-regulated in obese subjects after weight loss at 5- and 12-month time points (Supplemental Figure 4E).

Correlations between SIRT1 and total PARP activity and clinical parameters

Given the role of SIRT1 and PARPs in the regulation of whole-body energy metabolism, we also examined the correlations of the SAT *SIRT1* expression and the total PARP activity with adiposity and insulin sensitivity. SAT *SIRT1* mRNA correlated negatively with fat percentage (Figure 4A), liver fat (Figure 4B), and HOMA index (Figure 4C) and positively with Matsuda index (Figure 4D) at baseline in all subjects. However, no correlations were observed for glucose uptake of SAT and *SIRT1* mRNA. SAT *SIRT1* mRNA was not correlated with BAT glucose uptake at baseline (Figure 4E), but the change in SAT *SIRT1* mRNA between baseline and 5 months was positively correlated with the change in BAT glucose uptake between baseline and 5 months (Figure 4F). No other correlations were found between *SIRT1* mRNA and the clinical variables. In contrast to *SIRT1* mRNA, total PARP activity only correlated positively with fat percentage at baseline in obese subjects (Figure 4G). Additionally the inflammation pathway expression was negatively correlated with *SIRT1* expression (Supplemental Figure 4D), but not with *PARP1* expression (Supplemental Figure 4C), whereas oxidative stress pathway was positively correlated with *PARP1* expression (Supplemental Figure 4F) and negatively with *SIRT1* (Supplemental Figure 4G).

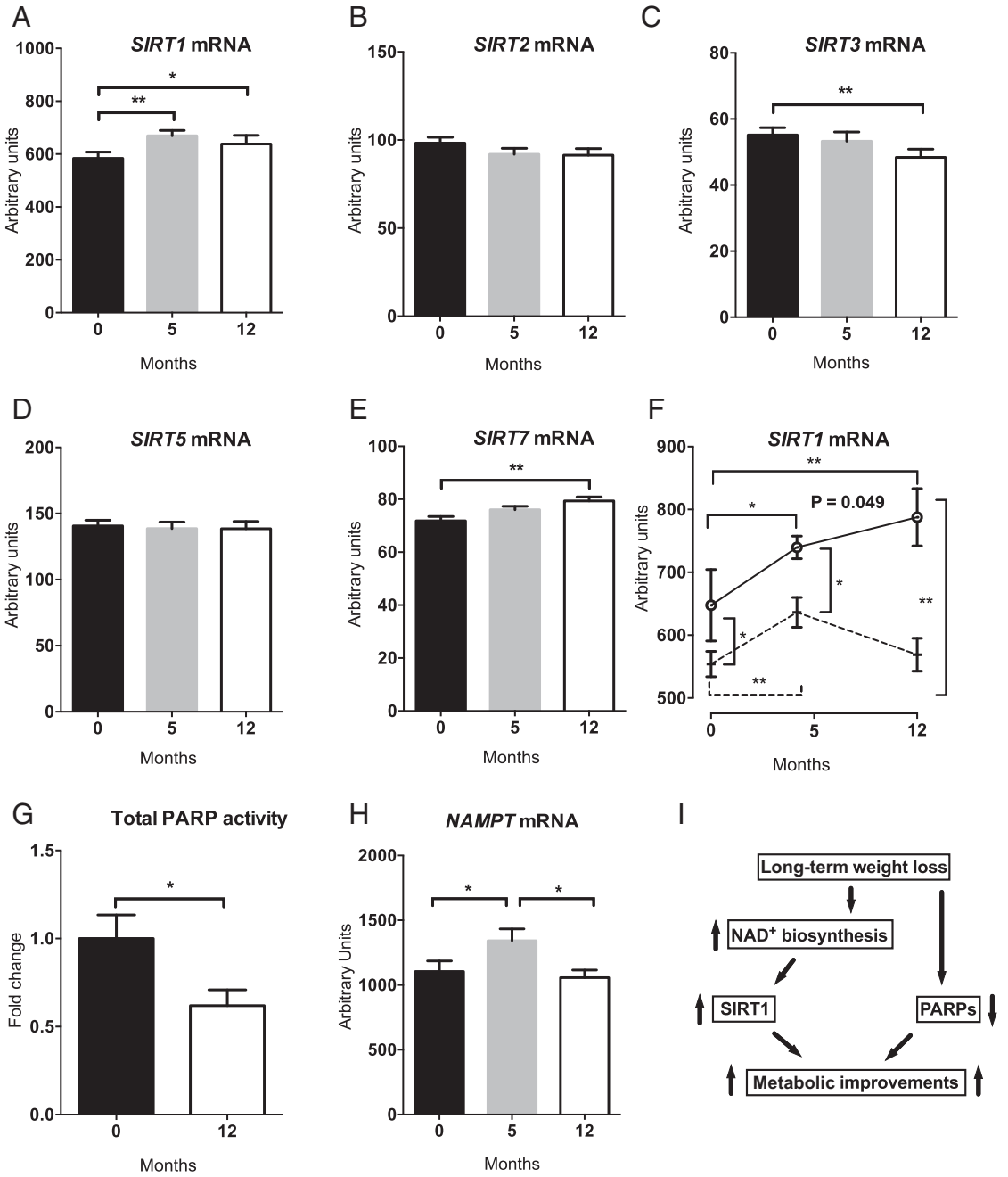

Figure 3. The mRNA levels of *SIRT*s, *NAMPT*, and total PARP activity in SAT during weight loss in obese subjects. *SIRT1* (A), *SIRT2* (B), *SIRT3* (C), *SIRT5* (D), and *SIRT7* (E) mRNA levels in all study subjects (n = 19) during the 12-month weight-loss intervention. Arbitrary units denote Affymetrix mRNA values after normalization. *SIRT1* mRNA levels in the 2 separate groups of weight loss subjects (F). The solid line represents the continuous weight losers (n = 6), and the dashed line represents the weight regainers (n = 13). Total PARP enzyme activity (n = 13 of all subjects) (G) and *NAMPT* mRNA levels (n = 18) (H) during weight loss in SAT in obese subjects. Suggested effects of long-term weight loss on improved metabolism via SIRT1 and PARPs (I). SIRT1 is activated via increased NAD^+ availability due to elevated NAD^+ biosynthesis and lowered PARP activity. Weight loss through energy shortage and the amelioration of oxidative stress decreases PARP activity. Data shown as mean ± SE. *, $P < .05$; **, $P < .01$; ***, $P < .001$. *P* values were calculated with repeated measures ANOVA or paired *t* tests.

Discussion

SIRTs and PARPs are recognized as 2 important NAD^+-dependent enzyme families with opposing metabolic effects. However, little is known about their biology in human SAT in obesity and after long-term weight loss. Here, we observed that mRNA levels of *SIRT1*, *SIRT3*, *SIRT7*, and the rate-liming gene in NAD^+ biosynthesis, *NAMPT*, were significantly lower, whereas the enzyme activity of PARPs was significantly higher in SAT of healthy obese subjects than in SAT of reference subjects. Using all subjects, we found a significant negative correlation of body fat percentage with SAT *SIRT1* mRNA levels and a positive correlation with SAT total PARP activity. Of the

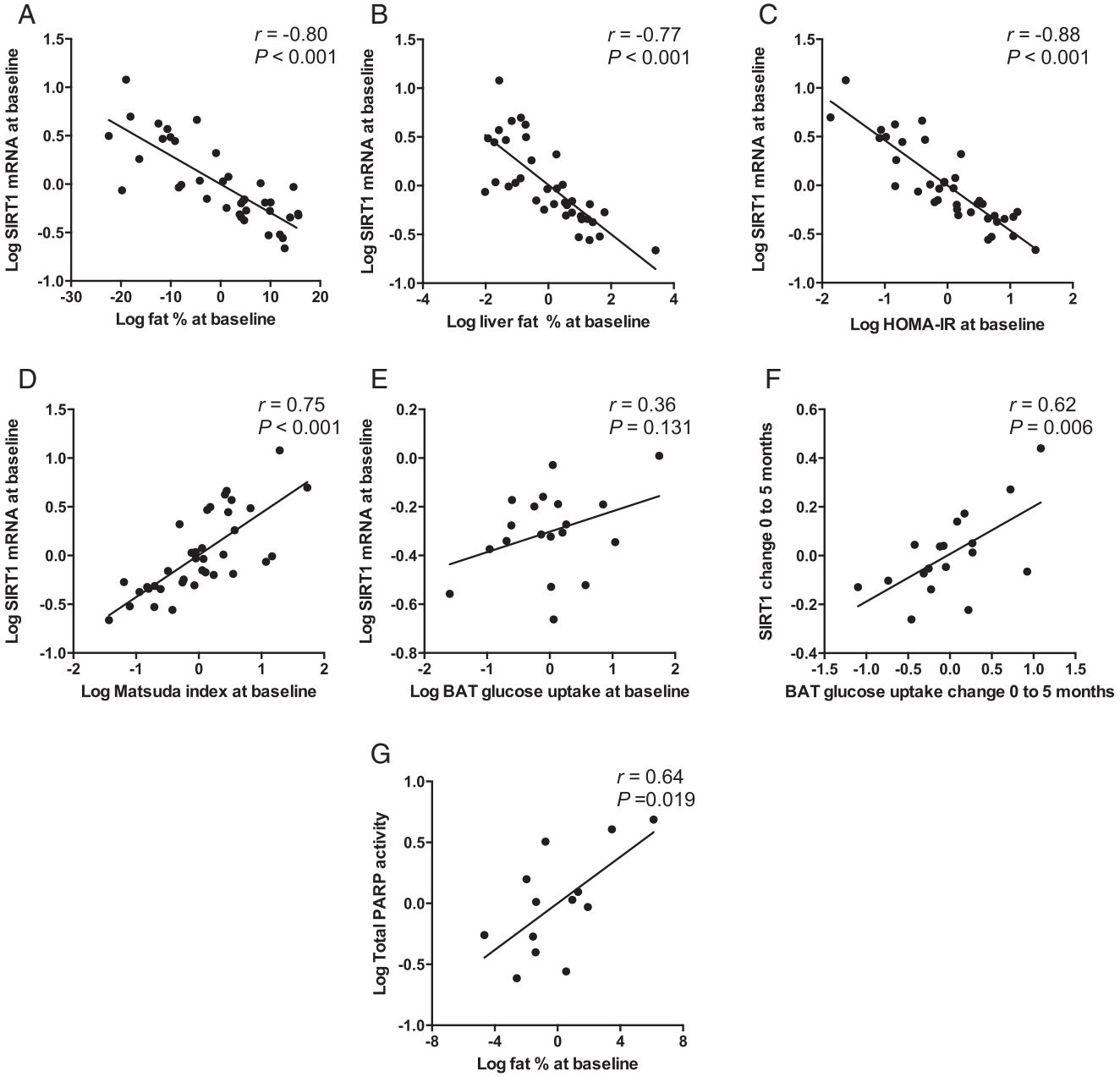

Figure 4. Partial regression plots of SAT *SIRT1* and PARP activity with select clinical parameters, adjusting for age and sex. The correlations between *SIRT1* microarray mRNA levels and fat percentage (A), liver fat (B), HOMA index (C) Matsuda index (D), BAT glucose uptake at baseline (E), and the correlation between the changes in *SIRT1* microarray mRNA levels and BAT glucose uptake between 0 and 5 months (F). The correlation between total PARP activity and fat percentage at baseline (G); n = 13–38.

SIRTs, the effect of weight loss was the most pronounced on *SIRT1*: its expression increased upon weight loss and reverted back after weight regain. Consistent with the increased *SIRT1* expression, *NAMPT* expression increased, whereas total PARP activity decreased after a long-term weight loss. Together, these data suggest that human SAT actively responds to the changes in NAD^+ biosynthesis. Moreover, weight loss increases NAD^+/SIRT pathway expression and reduces enzymatic activity of PARP.

In this study, we examined whether clinically healthy obese subjects displayed perturbations in NAD^+/SIRT pathway and PARP activity in SAT before the emergence of obesity-related metabolic abnormalities. We documented a significant down-regulation of *SIRT1*, *SIRT3*, and *SIRT7* in obese compared with reference subjects. This finding is in line with studies in mice, where the expression of *Sirt1* (7) and *Sirt3* (27) are down-regulated after HFD. Previous data in humans are scarce and restricted to *SIRT1* expression, which is shown to be reduced in SAT with increased BMI (28, 29). Compared with lean individuals, our obese subjects also exhibited significantly higher enzyme activities of PARPs despite of

unchanged *PARP1* and *PARP2* gene expression levels. However, it has been published that PARP activity does not reflect the main isoform, *PARP1*, gene expression levels (30). As expected based on previous studies in humans (31, 32), our obese subjects showed lower gene expression of *NAMPT*, the rate-limiting enzyme in the main mammalian biosynthetic route to NAD^+ (6). In mice, HFD is known to reduce *Nampt* expression (8) and to activate PARPs (6) with a subsequent decrease in NAD^+ levels in white adipose tissue. Therefore, the observed down-regulation of *SIRT1*, *SIRT3*, and *SIRT7* in obese subjects could be partially caused by a reduced NAD^+ availability (Figure 2H), because at least the transcription of *Sirt1* is regulated by NAD^+ levels (33).

CR and weight loss have been shown to correlate positively with the improvements in metabolic parameters in mice (34) and humans (35), respectively. Indeed, in the present study, weight loss resulted in better insulin sensitivity and lower lipid values, BP and less oxidative stress. The metabolic benefits of CR have been suggested to be mediated by SIRT1 in several animal studies (19, 36). In humans, the effect of CR on SAT *SIRT1* expression has so far been studied only after fasting (37) and short-term weight-loss interventions in nondiabetic morbidly obese subjects (21). We demonstrate here for the first time that the human SAT *SIRT1* is clearly responsive to a prolonged energy shortage as the long-term weight-loss up-regulated *SIRT1* in SAT. Notably, the 2 groups, continuous weight losers and weight maintainers, differed significantly for their SAT *SIRT1* expression; it increased progressively in the former and reverted back to baseline after weight regain in the latter group. The fact that *SIRT1* mRNA levels were higher in the continuous weight loss group already at the beginning of the study raises the question, if baseline *SIRT1* activity could predict better weight-loss outcome in humans. This hypothesis is new and needs to be confirmed in future studies.

In addition to expression of SIRTs, CR and fasting are known to also induce NAD^+ biosynthesis by elevating *Nampt* expression 2- to 3-fold in animals (5, 38). Consistent with this, the expression of *NAMPT* in our obese subjects was significantly up-regulated at the time point with the most prominent weight loss and energy intake decline and not after the nadir. Hence, CR and weight loss seem to boost NAD^+ biosynthesis in human SAT, but weight regain probably compromises this pathway. In contrast to NAD^+ biosynthesis, the long-term weight loss significantly decreased NAD^+ consumption via PARPs, because total PARP activity was significantly decreased in SAT, similarly to what has been observed after fasting in mice (6). In addition to the nutritional status, the amelioration of the oxidative stress could suppress PARP activity upon weight loss. As a whole, we conclude that prolonged CR elevates NAD^+ availability by stimulating NAD^+ biosynthesis and decreasing PARP activity, which may drive at least SIRT1 transcription and activation (Figure 3I).

Recently, SIRT1 has emerged as an important regulator of insulin sensitivity also in humans (12, 39). Our results reinforce this notion, because SAT *SIRT1* expression correlated positively with Matsuda index and negatively with HOMA index at baseline in our healthy subjects. One potential mechanism explaining increased insulin sensitivity upon SIRT1 activation is improved mitochondrial function (6), as better mitochondrial capacity can enhance glucose utilization. Along this line, we found that the more the SAT *SIRT1* expression increased, the more the BAT glucose uptake increased during weight loss between the baseline and the 5-month time period. During this period weight decreased on average most dramatically. This could indicate that the subjects who are able to increase *SIRT1* in SAT also experience improved metabolic efficiency in BAT upon weight loss. Interestingly, BAT has been suggested to be the key tissue mediating the beneficial effects of SIRT1 on glucose homeostasis (40). Overall, our results reveal that high SAT *SIRT1* expression associates with improvements in whole-body insulin sensitivity and glucose utilization in BAT in humans.

The main limitation of our study was the small number of subjects investigated. Thus, our statistical power to detect significant changes is limited in the smallest subgroup analyses, leading to false negative results (type II errors) when small or medium effects are present. Nonsignificant results should not be interpreted as proving there is no effect. However, regarding "very large" effects, such as the observed increases in SIRT1 expression in the continuous weight loss group, the statistical power was sufficient.

In this study, we demonstrated that clinically healthy obese subjects showed decreased expression of the NAD^+/SIRT pathway, whereas the activation of PARPs possibly reflects poor ongoing dietary habits. Intriguingly, CR clearly elevates NAD^+/SIRT1 pathway expression and suppresses PARP activity and oxidative stress levels along with the improvements in metabolic parameters. In summary, SAT NAD^+/SIRT pathway and PARPs seem to be responsive to the energy content of the diet in humans and CR can be used to induce the opposing effects on the NAD^+/SIRT pathway and PARPs to promote metabolic health.

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