*Original Research*

# Sex differences in muscle SIRT1 and SIRT3 and exercise + weight loss effects on muscle sirtuins

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### Impact Statement

Aging, overweight, and obesity are associated with metabolic disorders, including insulin resistance and type 2 diabetes. The sirtuins, SIRT1 and SIRT3, respond to metabolic change. Our new findings indicate sex differences in the skeletal muscle gene expression of these sirtuins, but changes with long duration of exercise training and weight loss are not different by sex. This new information from our study may show that SIRT1 and SIRT3 regulation may be foci to pursue in the treatment of certain diseases in aging.

### Abstract

The sirtuins, SIRT1 and SIRT3, are involved in the control of cellular processes to maintain metabolic homeostasis. The purpose of this study was to determine the effects of a 6-month aerobic training + weight loss program and hyperinsulinemia on SIRT1 and SIRT3 expression in skeletal muscle and to compare their expression between men and women. Thirty-five adult men ($n = 18$) and postmenopausal women ($n = 17$), (X ± SEM, age: 61 ± 1 years, BMI: 31.3 ± 0.7 kg/m$^2$) completed 6 months 3×/week of aerobic exercise and 1×/week dietary instruction to induce weight loss (EX + WL). Participants had a $VO_2$max test, vastus lateralis muscle biopsies at baseline and 2 h into a hyperinsulinemic-euglycemic clamp, a total body dual-energy X-ray absorptiometry scan, and abdominal computed tomography scan. Skeletal muscle SIRT1, SIRT3, and PGC1-α mRNA expression were quantified by qRT-PCR. Skeletal muscle SIRT1 and SIRT3 mRNA expression are higher in women than men ($P < 0.005$). Body weight, body fat, and abdominal obesity decreased and $VO_2$max and glucose utilization (M) increased after EX + WL ($P < 0.001$). Basal SIRT1 decreased following EX + WL ($P < 0.05$). This change in basal SIRT1 was not related to changes in $VO_2$max, M or fat mass, nor was it different by gender. Insulin stimulation increased SIRT1 expression ($P < 0.001$) and PGC1-α expression ($P < 0.01$) following EX + WL (insulin-basal post). Sex differences in the levels of these sirtuins did not affect changes with EX + WL. Skeletal muscle SIRT1 decreases after a long-term combined exercise and weight loss program in middle-aged and older adults.

**Keywords:** Exercise, skeletal muscle, metabolism, glucose, nutrition, obesity

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## Introduction

Being overweight or obesity increases the likelihood for type 2 diabetes, whereas lifestyle interventions which reduce body mass is associated with a reduction the development of developing type 2 diabetes. Aging is related to a systemic decrease of nicotinamide adenine dinucleotide (NAD), which is associated with modifications in NAD+-consuming enzyme activity, mitochondrial dysfunction, DNA damage, and inflammation.[1] Sirtuins are NAD+-dependent deacylases and ADP ribosyltransferases which use NAD+.[1] The sirtuin family has seven members (SIRT1-7); SIRT1 is located in the nucleus and cytoplasm and SIRT3 in the mitochondria. Both SIRT1 and SIRT3 exhibit deacetylase activity[2] and control critical cellular processes to reduce cellular damage and inflammation as well as to maintain metabolic homeostasis.[3]

The production of ATP in skeletal muscle is necessary during physical activity and involves electron transfer in the mitochondrial electron transport chain. NAD is key to this transfer and the fact that SIRT1 and SIRT3 are dependent on NAD+ directly links their activity to the metabolic status of the cell.[2] SIRT1 influences skeletal muscle metabolism through deacetylation of peroxisome proliferator-activated receptor gamma coactivator-1-α (PGC1-α), considered a master regulator of genes involved in metabolism, mitochondrial biogenesis, and energy expenditure.[4,5] SIRT3 and PGC1-α rise in response to caloric restriction and exercise in animal models.[6–8] Although acute exercise may induce increases in muscle SIRT1 but not muscle SIRT3, several bouts of aerobic exercise increase muscle SIRT3 expression.[9] It remains unclear as to the impact of long-term exercise training with loss of body weight on skeletal muscle sirtuins.

SIRT1 reacts to fasting and stimulates fatty acid oxidation in the liver through the activation of PPARα. Moreover, SIRT3 deacetylates and activates mitochondrial enzymes implicated in fatty acid β-oxidation, amino acid metabolism, the electron transport chain, and antioxidant defenses.[2] PGC1-α modulates hepatic gluconeogenesis and is also induced with fasting.[6] We hypothesize that sirtuin levels are related to basal fatty acid oxidation, and that hyperinsulinemia and EX + WL will change SIRT1 and SIRT3 levels. However, there is a lack of knowledge of basal and hyperinsulinemic SIRT1 and SIRT3 expression and association with insulin sensitivity in older adults. Finally, we have previously shown higher fitness levels in men and smaller body fat percentage than women of comparable BMI and fat mass,[10] as well as greater insulin resistance in older men than women even after controlling for abdominal fat[11] suggesting that other metabolic factors influence differentiations in insulin sensitivity by sex. Furthermore, others report gender differences in serum SIRT1[12] which may explain gender differences in atherosclerotic cardiovascular disease[13] suggesting the hypothesis that there are sex differences in sirtuins in skeletal muscle. We sought to examine how 6-month EX + WL and hyperinsulinemia effect muscle SIRT1 and SIRT3 expression and to compare by sex.

## Materials and methods

### Participants

Participants were included if age > 50 years, body mass index (BMI) > 25 kg/m$^2$, weight-stable (<2.0 kg weight-change in past year), sedentary (<20 min of aerobic exercise 2×/week), nonsmokers, no known diabetes, did not demonstrate cancer, liver, renal or hematological disease, or other medical disorders by medical history and physical exam. A exercise test on the treadmill excluded those with asymptomatic coronary artery disease. Women demonstrated lack of menses >1 year. Thirty-five participants (18 men and 17 women, 7 African American, 28 Caucasian) met study criteria. We previously published detailed study procedures and results of EX + WL[10,14] but the muscle gene expression of sirtuins and PGC-1α were not previously reported. The study took place at Baltimore Veteran Affairs Medical Center and University of Maryland Baltimore whose Institutional Review Board approved the study. Written informed consent was obtained from every participant.

### $VO_2$max

$VO_2$max was measured using a continuous exercise stress test on a treadmill as previously described.[10,14]

### Height, weight, DXA, and CT

As previously described, height (cm), weight (kg), and body composition were measured using whole body dual-energy X-ray absorptiometry (DXA) (Prodigy, LUNAR Radiation Corp., Madison, WI) and computed tomography (CT) scan at $L_4$–$L_5$ (Siemens Somatom Sensation 64 Scanner, Fairfield, CT).[10,14] MIPAV (NIH Image Analysis Program) program was utilized to measure visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas. Data are missing for one person for the DXA and five participants for the abdominal CT scan.

### Hyperinsulinemic–euglycemic clamp and oxidative metabolism

The clamp and oxidative metabolism were conducted when the participant reported to the laboratory after a 12 hr overnight fast and two weeks weight stability of less than 2%. A eucaloric diet (carbohydrate of 50–55% and >150 g, protein of 15–20%, and fat of ⩽30%) was provided to participants for 2 days before both clamps by a registered dietician to control nutrient intake. In addition, participants were weight-stable (<2%) prior to repeat testing with 36–48 h after exercise. The 3-h hyperinsulinemic–euglycemic clamp technique[15] at 80 mU m$^{-2}$ min$^{-1}$ was performed as previously described with determination of peripheral tissue sensitivity to exogenous insulin (M), M/I, an index of insulin sensitivity, plasma glucose and insulin levels.[10,14] Three men did not undergo the glucose clamp. The participants collected a 24-h urine before each clamp. Glucose, fat, and protein oxidation were determined as described.[10,14]

### Skeletal muscle biopsies and RT-PCR

Needle biopsies of the vastus lateralis were conducted under local anesthesia and muscle handled as previously described.[10,14] RNA isolation and Quantitative Real-Time PCR (qPCR) of SIRT1, SIRT3, and PGC1-α gene expression was performed in our lab[11] with ThermoFisher primer/probes of SIRT1 (Assay ID: Hs01009003), SIRT3 (Assay ID Hs00202030), and PGC1-α (Assay ID Hs01016721) with normalization to 36B4 mRNA (Assay ID: Hs99999902).

### Aerobic exercise and weight loss intervention

Adults were enrolled in a 6-month, three times per week exercise program that was of moderate-to-high intensity combined with dietary induced weight loss (EX + WL) during 2001–2008. Before beginning the intervention, participants did a food record for 7 days, convened with a registered dietitian 1 day/week for 6–8 weeks, and were taught how to maintain a weight-stable, Therapeutic Lifestyle Changes (TLC) diet.[16] The exercise prescription began at ~50–60% heart rate reserve for 20–30 continuous minutes, progressed to 50 min at 3–4 weeks, and remained at 50 min for the duration of the program with progression of intensity >60–80% $VO_2$max. Chest-strap heart rate monitors (Polar Electro Inc., Lake Success, NY) were used to monitor exercise intensity during the sessions which were directed by exercise physiologists. A registered dietician led classes for weight loss one time per week for 6 months where instructions were provided to participants to follow a reduction in intake of 500 kcal/d. The American Diabetes Association exchange list system was used to monitor compliance through 7-day food records (or 24-h recalls).

### Statistics

Descriptive statistics including mean, standard error of the mean, and range were examined with SPSS (PASW Statistics, Version 22, Chicago, IL). Paired *t*-tests were conducted to

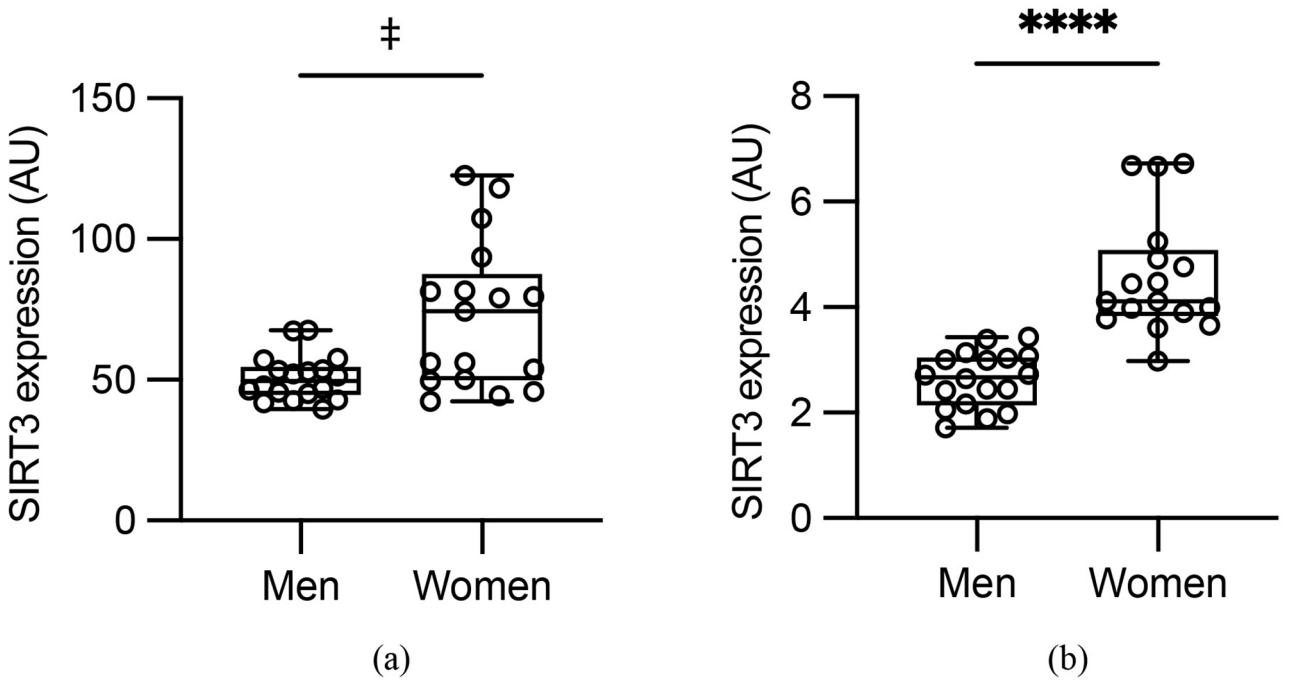


**Figure 1.** (a) Relative skeletal muscle SIRT1 mRNA expression at baseline in women ($n=17$) and men ($n=17$) ($^{‡}P<0.005$, between groups) and (b) relative skeletal muscle SIRT3 mRNA expression at in women ($n=17$) and men ($n=18$) ($^{****}P<0.0001$, between groups).

assess differences among pre-intervention and post-intervention in outcome variables. Main outcome variables were assessed for significant linear relationships by Pearson's correlation coefficient. Variables with more than one significant linear relationship were then examined by multiple regression, controlling for gender when relevant. The data (mean ± SEM) with a $P<0.05$ was considered significant.

## Results

### Baseline

Our study group of men and women whom we have shown to have metabolic differences[10] in that insulin sensitivity and glucose utilization were significantly higher in women than men. Therefore, we first compared the expression of SIRT1, SIRT3, and PGC1-α genes between genders. Basal SIRT1 and 3 expressions were significantly higher in women compared to men (Figure 1). We then examined correlations among the genes as well as their relationships with insulin sensitivity and basal fat oxidation. Basal SIRT1 mRNA was associated with basal muscle SIRT3 mRNA ($r=0.88$, $P<0.001$, Figure 2(a)). Basal skeletal muscle SIRT1 mRNA was associated with PGC1-α ($r=0.48$, $P<0.01$, Figure 2(b)) and SIRT3 mRNA was associated with PGC1-α ($r=0.36$, $P=0.05$, Figure 2(c)). Basal SIRT3 expression was inversely related to $VO_2$max ($r=-0.50$, $P<0.005$), and SIRT1 tended to be related to $VO_2$max ($r=-0.33, <0.06$). Basal SIRT1 and SIRT3 were directly related to fat mass ($r=0.54$, $P=0.001$; $r=0.46$, $P=0.005$, respectively) and percent body fat ($r=0.60$, $P<0.001$; $r=0.74$, $P<0.001$, respectively, Figure 2(d) and (e)). Basal PGC1-α was related to fat mass ($r=0.45$, $P<0.05$) and percent body fat ($r=0.39$, $P<0.05$, Figure 2(f)). In the examination of the relationships between basal muscle expression and visceral fat, and subcutaneous abdominal fat, the relationships were not significant for visceral fat (SIRT1 $r=0.193$, $P=0.17$, SIRT3 $r=0.068$, $P=0.70$ and PGC1-α $r=0.250$, $P=0.26$). These relationships were, however, significant for subcutaneous abdominal fat (SIRT1 $r=0.380$, $P<0.05$, SIRT3 $r=0.393$, $P<0.05$; and PGC1-α $r=0.444$, $P<0.05$). Basal SIRT1 tended to be related to fat oxidation ($r=0.40$, $P<0.06$). Neither variable was related to age. Multiple regression analyses showed that only fat mass significantly predicted SIRT1 and SIRT3 expressions ($P<0.005$), controlling for $VO_2$max, M, and gender (adjusted $R^2=41\%$ and 67%, respectively). Basal PGC1-α expression was only related to fat mass ($r=0.45$, $P<0.05$). The effect of insulin on SIRT1 and SIRT3 and PGC1-α expressions were not different between genders. Insulin tended to increase SIRT1 expression pre EX + WL ($P<0.09$, Figure 3(a)), did not significantly change SIRT3 (Figure 3(b)), and significantly increased PGC1-α expression pre EX + WL ($P\leqslant 0.001$, Figure 3(c)).

### EX + WL effects

Physical and metabolic characteristics before and after EX + WL are presented in Table 1. As previously reported,[14] body weight, fat mass, percent body fat, visceral fat, and subcutaneous abdominal fat decreased after EX + WL ($P<0.001$). There was no change in FFM (Table 1). $VO_2$max (L/min) increased 21% after EX + WL ($P<0.001$). Glucose utilization and insulin sensitivity increased after EX + WL (both $P<0.0001$). Basal fat and carbohydrate oxidation did not significantly change with EX + WL.

Basal SIRT1 decreased following EX + WL ($P<0.05$, Figure 2(a)). This change in basal SIRT1 was not associated with changes in $VO_2$max, M or fat mass, nor was it different

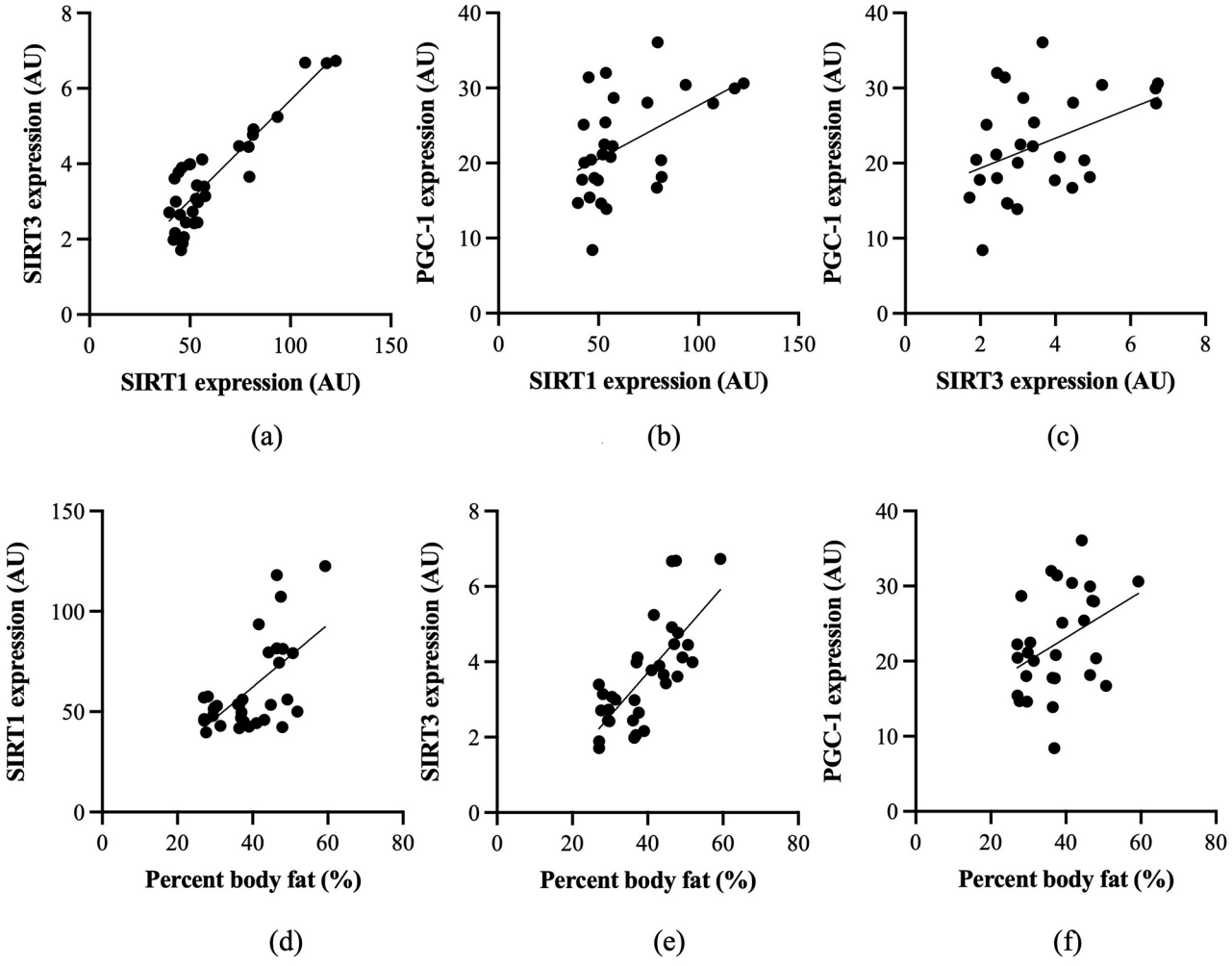


**Figure 2.** (a) The relationship of basal skeletal muscle SIRT1 levels with muscle SIRT3 levels in older men and women ($r = 0.88$, $P < 0.001$). (b) The relationship of basal skeletal muscle SIRT1 levels with muscle PGC1-α levels ($r = 0.48$, $P < 0.01$), (c) The relationship of basal skeletal muscle SIRT3 with muscle PGC1-α levels, (d) The relationship of percent body fat with basal muscle SIRT1 levels ($r = 0.60$, $P < 0.001$), (e) The relationship of percent body fat with basal muscle SIRT3 ($r = 0.74$, $P < 0.001$), (f) The relationship of percent body fat with basal muscle PGC1-α levels ($r = 0.39$, $P < 0.05$).

by gender. We then examined changes in regional fat composition and changes in basal muscle expression. Changes in visceral and subcutaneous abdominal fat were not significantly associated with changes in basal SIRT1, SIRT3, or PGC1-α mRNA (data not shown). Insulin stimulation increased SIRT1 expression post EX + WL ($P < 0.0001$, Figure 3(a)), did not change SIRT3 expression (Figure 3(b)) post EX + WL and increased PGC1-α expression post EX + WL ($P < 0.005$, Figure 3(c)) (insulin-basal post). The change in insulin's effect on SIRT1 expression after EX + WL (insulin-basal post) was inversely related to M following EX + WL ($r = -0.38$, $P < 0.05$), but not related to $VO_2$max or fat mass following EX + WL. The change in insulin effect on PGC1-α expression post EX + WL was not related to M, $VO_2$max or fat mass following EX + WL. The change in insulin action on SIRT1 following EX + WL (insulin-basal pre versus insulin-basal post) tended to be significant ($P = 0.07$).

## Discussion

There are physiologically relevant sex differences in the levels of SIRT1 and SIRT3, namely, a 30–40% higher level in women than men. Our analyses indicate that this is independent of age, fitness and insulin sensitivity and appears to be driven by body fat. Skeletal muscle SIRT1 and SIRT3 are an integral part of skeletal muscle glucose utilization in adults. The gene expression of SIRT1 decreases but SIRT3 is not altered after a long-term combined exercise and weight loss program, with improvements in insulin sensitivity.

Although serum SIRT1 activity levels have been shown in one study not to differ between men and women, levels peak in women in their thirties and men in their forties and thereafter decline with age in both sexes.[12] There is limited other information on age-related changes in serum SIRT levels in humans. Total protein expression of muscle SIRT1 and PGC-1α were reported to be similar in young men and women despite higher type I fibers in women.[17] An increase in SIRT1 content and/or activity modulates PGC-1α promoting fiber type conversion.[18] Furthermore, expression of PGC-1α and SIRT1 is increased in oxidative (type I) compared to glycolytic (type II) muscle fibers.[18] Thus, it is possible that sex differences in fiber type are important in sirtuin levels but this was not measured in our muscle samples. Our results show that the muscle mRNA expression of SIRT1 and SIRT3

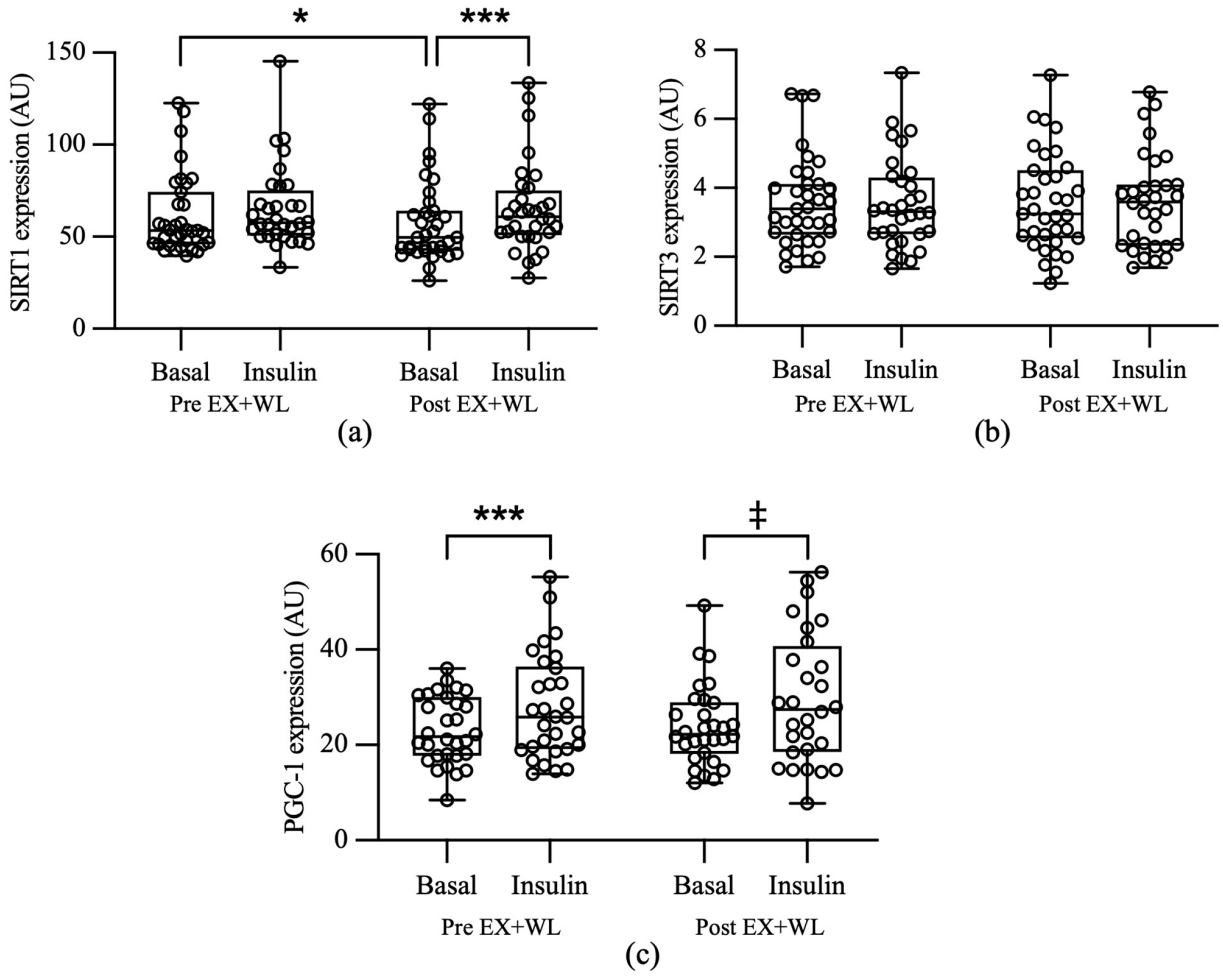


**Figure 3.** (a) Skeletal muscle SIRT1 levels during basal (solid) and insulin-stimulated (striped) conditions pre and post aerobic exercise + weight loss (AEX + WL). *$P < 0.05$, ****$P < 0.0001$. (b) Skeletal muscle SIRT3 levels during basal (solid) and insulin-stimulated (striped) conditions pre and post aerobic exercise + weight loss (AEX + WL). (c) Skeletal muscle PGC1-α levels during basal (solid) and insulin-stimulated (striped) conditions pre and post aerobic exercise + weight loss (AEX + WL). ***$P \leqslant 0.001$, ‡ $P < 0.005$.

**Table 1.** Subject characteristics, body composition, and metabolic measurement before and after 6 months of aerobic exercise + weight loss (AEX + WL).

| Characteristic | Before AEX + WL | After AEX + WL |
|---|---|---|
| Age (years) | 61.2 ± 1.1 | |
| Weight (kg) | 91.6 ± 2.8 | 84.6 ± 2.7‡ |
| BMI (kg/m$^2$) | 31.3 ± 0.7 | 28.5 ± 0.7‡ |
| Percent body fat | 39.2 ± 1.5 | 34.994 ± 1.7‡ |
| Fat mass (kg) | 35.8 ± 1.8 | 29.8 ± 1.8‡ |
| Fat-free mass (kg) | 55.9 ± 2.2 | 55.4 ± 2.3 |
| Visceral fat area (cm$^2$) | 160.1 ± 14.7 | 127.5 ± 12.5‡ |
| Subcutaneous abdominal fat area (cm$^2$) | 356.6 ± 19.0 | 301.2 ± 21.3‡ |
| $VO_2$max (mL/kg/min) | 21.8 ± 1.0 | 26.81 ± 1.3‡ |
| $VO_2$max (L/min) | 2.00 ± 0.11 | 2.26 ± 0.12‡ |
| Fasting glucose (mmol/L) | 5.41 ± 0.10 | 5.25 ± 0.09 |
| Fasting insulin (pmol/L) | 87 ± 7 | 69 ± 5‡ |
| Basal fat oxidation (μmol/kg $_{FFM}$/min) | 6.96 ± 0.44 | 7.25 ± 0.53 |
| Basal carbohydrate oxidation (μmol/kg $_{FM}$/min) | 5.66 ± 1.01 | 4.78 ± 1.45 |
| Glucose utilization (mg/kg/min) | 5.13 ± 0.31 | 6.60 ± 0.33‡ |
| Glucose utilization (μmol/kg $_{FFM}$/min) | 48.01 ± 3.29 | 56.50 ± 2.86‡ |
| Insulin sensitivity (mg/kg/min/min/pM) | 0.026 ± 0.002 | 0.036 ± 0.002‡ |
| Insulin sensitivity (μmol/kg $_{FFM}$/min/pM) | 0.044 ± 0.004 | 0.054 ± 0.003‡ |

BMI: body mass index.
Values are mean ± SEM.
Significant different before and after the intervention: ‡$P < 0.001$.

in females is significantly greater in males and could suggest that other gender-related factors are contributing factors to differences by sex in these sirtuins.

We provide the novel finding that skeletal muscle SIRT1 and PGC1-α mRNA expression, but not SIRT3 expression, increase during in vivo hyperinsulinemia. There are few studies that have investigated the controlled effect of exogenous insulin infusion on the expression of these three genes. In young normal-weight, overweight as well as obese participants, hyperinsulinemia increased muscle expression of SIRT1 but muscle SIRT1 did not relate to insulin sensitivity.[19] One study using a hyperinsulinemic-euglycemic clamp in rats reported no changes in PGC-1α mRNA expression with insulin alone or combined with IL-6 infusion.[20] We are unaware of any other studies which conducted two muscle biopsies to examine changes in these sirtuins during a clamp. Our results indicate that SIRT1 as well as PGC-1α mRNA, but not SIRT3, increases with hyperinsulinemia which may reflect their roles in metabolic and energy status.

Overexpression of SIRT1 improves both glucose tolerance and insulin release from β-cells after glucose administration in mice.[21,22] Activated SIRT1 improves the insulin sensitivity of liver, skeletal muscle, and adipose tissue, and enhances fat oxidation in human and mice tissue.[23–26] In adults, small molecule activators of SIRT1 augment insulin sensitivity.[27] Our data show that basal muscle SIRT1 and SIRT3 mRNA expressions are correlated with each other, and both are correlated to glucose utilization and insulin sensitivity. Basal SIRT1 mRNA level decreases after EX + WL, but the change between basal and insulin-stimulated levels increases, suggesting SIRT1 contributes to insulin sensitivity. Yet, we did not find a relationship between the improvements in M and changes in SIRT1 levels in our study sample. In a small sample (total $n = 9$) of young adults (mean age = 23 years) of three females and six males, total muscle SIRT1 activity (31%) and activity per SIRT1 protein (58%) rose but SIRT1 protein declined (20%) after high-intensity interval training of 6 weeks duration.[28] Because there was limited tissue available, we could not examine the activity and protein level of SIRT1 and SIRT3. It is possible that activity levels would have increased with EX + WL.

PGC-1α mRNA muscle expression is lower in patients with type 2 diabetes than obese controls[29] and insulin resistant states.[30,31] However, an improvement in insulin sensitivity by rosiglitazone treatment was not correlated with an upregulation of PGC-1α mRNA expression in type 2 diabetes.[29] Our findings indicate that exercise and weight loss improvements in insulin sensitivity were not associated with changes in muscle PGC-1α mRNA expression.

Exercise training can enhance mitochondrial oxidative activity and fat oxidation and improve insulin sensitivity via the SIRT1/PGC-1α axis.[32] SIRT1 in muscle increases PGC-1α transcriptional activity through deacetylation, thereby increasing mitochondrial content, skeletal muscle function, and metabolic health.[32] SIRT1 increases oxidation of fatty acids by activating PPAR-α through deacetylation of PGC-1α.[23] SIRT1 mRNA expression correlated with PGC-1α mRNA expression in skeletal muscle and basal fat oxidation in our study. SIRT1 and PGC-1α expression in skeletal muscle may be considered as a target for reducing obesity and improving metabolic health.

Exercise training increases SIRT3 expression associated PGC-1α upregulation.[33] High-intensity interval training of 12 weeks increases peripheral blood mRNA levels of PGC-1α in a small pilot study of young adults.[34] Aerobic training increases muscle SIRT3 protein level; SIRT3 was related to PGC-1α and percentage of body fat in sedentary obese male adolescents.[35] However, SIRT3 does not change after sprint-interval training with no correlation reported between SIRT3 and PGC-1α in skeletal muscle.[36] We also found that basal SIRT3 and PGC-1α mRNA expression in skeletal muscle did not change after EX + WL.

This study has some limitations and strengths worth mentioning. Given that our intervention combined exercise and weight loss, we are unable to discern the independent effects of either aerobic exercise or WL on sirtuin levels. To address this limitation, we added $VO_2$max and fat mass to all regression models to better understand the role of the interventions on the outcomes. Our study was also limited by the amount of muscle tissue obtained. Measurement of sirtuin protein levels or activity might provide additional in-sight as to the mechanism for the enhanced insulin sensitivity following EX + WL. A study sample that included adults with type 2 diabetes could inform relationships of sirtuins in more insulin resistant states. However, our methods of rigorous diet and center-based progressive exercise intervention, well controlled hyperinsulinemia during a glucose clamp, and measurement of sirtuins directly in skeletal muscle, are strengths of note.

Aerobic training and weight reduction improves fitness, lowers abdominal and total body fat, and improves insulin sensitivity with significant changes of SIRT1 expression in skeletal muscle in adults. The relationships between sirtuins and PGC-1α and with insulin sensitivity reflect that their regulation could be the objective for the management of metabolic diseases in aging and obesity.

#### AUTHORS' CONTRIBUTIONS

ASR designed the research, performed the experiments, collected and analyzed the data, and wrote the manuscript; GL performed the experiments and contributed to analysis of the data and writing of the manuscript.

#### DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

#### ETHICAL APPROVAL

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the University of Maryland, Baltimore.

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## REFERENCES

1. Amjad S, Nisar S, Bhat AA, Shah AR, Frenneaux MP, Fakhro K, Haris M, Reddy R, Patay Z, Baur J, Bagga P. Role of NAD(+) in regulating cellular and metabolic signaling pathways. *Mol Metab* 2021;**49**:101195
2. Kincaid B, Bossy-Wetzel E. Forever young: SIRT3 a shield against mitochondrial meltdown, aging, and neurodegeneration. *Front Aging Neurosci* 2013;**5**:48
3. Winnik S, Auwerx J, Sinclair DA, Matter CM. Protective effects of sirtuins in cardiovascular diseases: from bench to bedside. *Eur Heart J* 2015;**36**:3404–12
4. Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 2001;**413**:179–83
5. Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. *Mol Cell* 2001;**8**:971–82
6. Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 2001;**413**:131–8
7. Handschin C, Spiegelman BM. The role of exercise and PGC1alpha in inflammation and chronic disease. *Nature* 2008;**454**:463–9
8. Palacios OM, Carmona JJ, Michan S, Chen KY, Manabe Y, Ward JL 3rd, Goodyear LJ, Tong Q. Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging (Albany NY)* 2009;**1**:771–83
9. Vargas-Ortiz K, Perez-Vazquez V, Macias-Cervantes MH. Exercise and sirtuins: a way to mitochondrial health in skeletal muscle. *Int J Mol Sci* 2019;**20**:2717
10. Li G, Zhang H, Ryan AS. Skeletal muscle angiopoietin-like protein 4 and glucose metabolism in older adults after exercise and weight loss. *Metabolites* 2020;**10**:354
11. Ferrara CM, Goldberg AP, Nicklas BJ, Sorkin JD, Ryan AS. Sex differences in insulin action and body fat distribution in overweight and obese middle-aged and older men and women. *Appl Physiol Nutr Metab* 2008;**33**:784–90
12. Lee HJ, Yang SJ. Aging-related correlation between serum sirtuin 1 activities and basal metabolic rate in women, but not in men. *Clin Nutr Res* 2017;**6**:18–26
13. Shimabukuro M. SIRT1 and gender differences in atherosclerotic cardiovascular disease. *J Atheroscler Thromb* 2020;**27**:8–10
14. Ryan AS, Li G, Blumenthal JB, Ortmeyer HK. Aerobic exercise + weight loss decreases skeletal muscle myostatin expression and improves insulin sensitivity in older adults. *Obesity (Silver Spring)* 2013;**21**:1350–6
15. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;**237**:E214–123
16. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M, Wylie-Rosett J. Summary of American Heart Association Diet and lifestyle recommendations revision 2006. *Arterioscler Thromb Vasc Biol* 2006;**26**:2186–91
17. Guadalupe-Grau A, Rodriguez-Garcia L, Torres-Peralta R, Morales-Alamo D, Ponce-Gonzalez JG, Perez-Suarez I, Santana A, Al Calbet J. Greater basal skeletal muscle AMPKalpha phosphorylation in men than in women: associations with anaerobic performance. *Eur J Sport Sci* 2016;**16**:455–64
18. Pardo PS, Boriek AM. The physiological roles of Sirt1 in skeletal muscle. *Aging (Albany NY)* 2011;**3**:430–7
19. Stefanowicz M, Nikolajuk A, Matulewicz N, Karczewska-Kupczewska M. Adipose tissue, but not skeletal muscle, sirtuin 1 expression is decreased in obesity and related to insulin sensitivity. *Endocrine* 2018;**60**:263–71
20. Rotter Sopasakis V, Larsson BM, Johansson A, Holmang A, Smith U. Short-term infusion of interleukin-6 does not induce insulin resistance in vivo or impair insulin signaling in rats. *Diabetologia* 2004;**47**:1879–87
21. Moynihan KA, Grimm AA, Plueger MM, Bernal-Mizrachi E, Ford E, Cras-Meneur C, Permutt MA, Imai S. Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab* 2005;**2**:105–17
22. Bordone L, Motta MC, Picard F, Robinson A, Jhala US, Apfeld J, McDonagh T, Lemieux M, McBurney M, Szilvasi A, Easlon EJ, Lin SJ, Guarente L. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *Plos Biol* 2006;**4**:e31
23. Khan SA, Sathyanarayan A, Mashek MT, Ong KT, Wollaston-Hayden EE, Mashek DG. ATGL-catalyzed lipolysis regulates SIRT1 to control PGC-1alpha/PPAR-alpha signaling. *Diabetes* 2015;**64**:418–26
24. Cao Y, Jiang X, Ma H, Wang Y, Xue P, Liu Y. SIRT1 and insulin resistance. *J Diabetes Complications* 2016;**30**:178–83
25. Jeon JY, Choi SE, Ha ES, Lee HB, Kim TH, Han SJ, Kim J, Kim DJ, Kang Y, Lee KW. GLP1 improves palmitate induced insulin resistance in human skeletal muscle via SIRT1 activity. *Int J Mol Med* 2019;**44**:1161–71
26. Wang Q, Zhao B, Zhang J, Sun J, Wang S, Zhang X, Xu Y, Wang R. Faster lipid beta-oxidation rate by acetyl-CoA carboxylase 2 inhibition alleviates high-glucose-induced insulin resistance via SIRT1/PGC-1alpha in human podocytes. *J Biochem Mol Toxicol* 2021;**35**:e22797
27. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, Iffland A, Lavu S, Medvedik O, Sinclair DA, Olefsky JM, Jirousek MR, Elliott PJ, Westphal CH. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* 2007;**450**:712–6
28. Gurd BJ, Perry CG, Heigenhauser GJ, Spriet LL, Bonen A. High-intensity interval training increases SIRT1 activity in human skeletal muscle. *Appl Physiol Nutr Metab* 2010;**35**:350–7
29. Mensink M, Hesselink MK, Russell AP, Schaart G, Sels JP, Schrauwen P. Improved skeletal muscle oxidative enzyme activity and restoration of PGC-1 alpha and PPAR beta/delta gene expression upon rosiglitazone treatment in obese patients with type 2 diabetes mellitus. *Int J Obes (Lond)* 2007;**31**:1302–10
30. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, Mandarino LJ. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci* 2003;**100**:8466–71
31. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003;**34**:267–73
32. Williams CB, Gurd BJ. Skeletal muscle SIRT1 and the genetics of metabolic health: therapeutic activation by pharmaceuticals and exercise. *Appl Clin Genet* 2012;**5**:81–91
33. Palacios OM, Carmona JJ, Michan S, Chen KY, Manabe Y, Ward JL 3rd, Goodyear LJ, Tong Q. Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging* 2009;**151**:771–83
34. Vargas-Ortiz K, Perez-Vazquez V, Figueroa A, Diaz FJ, Montano-Ascencio PG, Macias-Cervantes MH. Aerobic training but no resistance training increases SIRT3 in skeletal muscle of sedentary obese male adolescents. *Eur J Sport Sci* 2018;**18**:226–34
35. Edgett BA, Bonafiglia JT, Baechler BL, Quadrilatero J, Gurd BJ. The effect of acute and chronic sprint-interval training on LRP130, SIRT3, and PGC-1α expression in human skeletal muscle. *Physiol Rep* 2016;**4**:e12879
36. Asilah Za'don NH, Amirul Farhana MK, Farhanim I, Sharifah Izwan TO, Appukutty M, Salim N, Farah NM, Fitri MLA. High-intensity interval training induced PGC-1 proportional, variant and AdipoR1 gene expressions and improved insulin sensitivity in obese individuals. *Med J Malaysia* 2019;**74**:461–7

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