Overexpression of SIRT1 in rat skeletal muscle does not alter glucose induced insulin resistance.

Amanda E. Brandon[1], Jennifer Tid-Ang[1], Lauren E. Wright[1], Ella Stuart[1], Eurwin Suryana[1], Nigel Turner[1,2], Gregory J. Cooney[1,2], Neil B. Ruderman[3], and Edward W. Kraegen[1,2].

[1]Diabetes and Obesity Program, Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, NSW 2010, Australia; [2]UNSW Medicine, University of New South Wales, Sydney, Australia; [3]Boston University School of Medicine, Boston, Massachusetts, USA.

Running Title:

Corresponding Author: Dr Amanda E. Brandon

Diabetes and Obesity Program,

Garvan Institute of Medical Research,

384 Victoria St.

Darlinghurst, NSW, 2010

AUSTRALIA

Phone: +61 2 9295 8310

Fax: +61 2 9295 8221

Email: a.brandon@garvan.org.au

Key Words: Glucose infusion, SIRT1, hyperglycaemia,

Abstract

SIRT1 is a NAD^+-dependent deacetylase enzyme thought to regulate metabolic pathways within cells in response to alterations in nutrient flux. In the current study we investigated whether acute changes in SIRT1 affect markers of muscle mitochondrial content and also determined if SIRT1 influenced muscle insulin resistance induced by acute glucose oversupply. Male Wistar rats were electroporated with either SIRT1 or a deacetylase inactive mutant of SIRT1 (H363Y) into one tibialis cranialis (TC) muscle with an empty vector in the other TC serving as a control. Animals then immediately underwent cannulation surgery. One week later, glucose was infused and hyperglycaemia was maintained at ~11mM. After 5 hours this protocol generates significant insulin resistance in skeletal muscle. Interestingly, overexpression of either SIRT1 or SIRT1 (H363Y) for 1 week did not change markers of mitochondrial content or function. SIRT1 or SIRT1 (H363Y) overexpression had no effect on the reduction in glucose uptake and glycogen synthesis in muscle in response to hyperglycemia. Therefore we conclude that acute increases in SIRT1 have little impact on mitochondrial function and that SIRT1 is not involved in the development of muscle insulin resistance that occurs after a hyperglycaemic insult.

208/300 Words

Introduction

Sirtuin 1 (SIRT1) is a NAD^+-dependent deacetylase enzyme with a large range of target proteins that are important for apoptosis, the cell cycle, circadian rhythms, mitochondrial function, and metabolism [1]. SIRT1 is thought to be nutritionally regulated and be responsible for the beneficial effects of calorie restriction [2,3]. Levels of SIRT1 are reportedly decreased with high fat feeding and may therefore have a role in lipid-induced insulin resistance [4]. Not surprisingly then, *in vitro* studies has shown that SIRT1 is down regulated under hyperglycaemic conditions in liver [5,6], endothelial [7,8], mesangial [9], corneal epithelial [10] and C2C12 muscle cells [11]. Rescuing the decrease in SIRT1 via pharmacological intervention (e.g. resveratrol) or protein overexpression can reverse the detrimental hyperglycaemic effect in these systems [8-10].

Using a model of hyperglycaemia (~11mM blood glucose) generated by a moderate intravenous glucose infusion into rats, we have shown previously that skeletal muscle insulin resistance consistently develops between 3 and 5h [12-14]. Interestingly, this insulin resistance developed prior to alterations in the insulin signaling pathway [12,13] but occurred in association of increased glycogen content and reduced AMPK activity [12,14]. In an attempt to further delineate the underlying mechanism(s) behind this insulin resistance, Saha *et al* [15] incubated EDL muscle strips in 5 v 25 mM glucose and found the lactate to pyruvate ratio, a surrogate marker of the NAD^+/NADH ratio (redox state), was increased. Although not significant, a decrease in SIRT1 protein levels (20%) was also found [15]. Thus, it is possible that SIRT1 may be playing a role in hyperglycemia induced insulin resistance. Hence, the aim of the current study was to investigate the role of SIRT1 in glucose-induced insulin resistance. We hypothesised that SIRT1 overexpression may prevent the development of insulin resistance in skeletal muscle *in vivo*.

Methods

Cell culture

All cell culture reagents are from Life Technologies (Auckland, NZ) unless otherwise stated. Mouse skeletal muscle cell line C2C12 myoblasts were maintained in 50/50 % Dulbecco's modified Eagle's medium (DMEM) and F12 supplemented with 10% FBS, 100 U/ml penicillin and 100 U/ml streptomycin (growth medium) at 37°C under a 5% CO_2 atmosphere. For transfection, the cells were grown on 6-well plates (Corning Inc., NY, USA) in 2 ml of growth medium. Once at 90% confluency, cells were transfected (2μg) with either empty plasmid, a truncated but fully active form of SIRT1, or a version of the SIRT1 construct that has a point mutation that renders it deacetylase inactive (SIRT1 (H363Y); kind gifts from Aimin Xu [16]) using the X-tremeGENE HP DNA transfection reagent (Roche, Mannheim, Germany) and opti-MEM reagent. Cells were collected 24h post transfection. Data presented are an average of 3 independent experiments.

Animals

All surgical and experimental procedures performed were approved by the Garvan Institute/St Vincent's Hospital Animal Ethics Committee and were in accordance with the National Health and Medical Research Council of Australia's guidelines on animal experimentation.

Adult male Wistar rats (Animal Resources Centre, Perth, Australia) were communally housed in temperature controlled (22 ± 0.5 °C) 12 h light-dark cycle rooms. Rats were fed *ad libitum* a standard chow diet (Rat Maintenance Diet; Gordon Specialty Feeds, Sydney, Australia). Rats were acclimatized for 1 week prior to surgery.

In Vivo Electroporation (IVE) and Surgical Procedures

After the acclimatization period, rats were electroporated as previously described [17-19]. Briefly, under anaesthesia, control and test muscles were pretreated for 2 h with 90 units of hyaluronidase to break down components of the extracellular matrix to improve transfection efficiency [20]. Either SIRT1, or SIRT1 (H363Y), was injected into the test (right) tibialis cranialis (TC), and empty plasmid was injected into the control (left) TC via 6 x 50ul injections. Both legs underwent an electroporation protocol consisting of one 800 V/cm, 100 ms pulse followed by four 80 V/cm, 100 ms pulses at 1 Hz. Immediately after the IVE, while still under anaesthesia, dual cannulation of both jugular veins was performed as described previously [12,21].

Glucose Infusion

Seven days after surgery, rats were randomly divided into treatment groups. After a basal blood sample was taken, a 50 % (w/v) glucose infusion commenced. Rats were infused for either 0 or 5h using a peristaltic roller pump (101U/R; Watson-Marlow, Falmouth, UK). Blood samples were taken every 30 min and the glucose infusion rate was altered to maintain blood glucose concentration at ~11 mM. Red blood cells from each sample were resuspended in heparinised saline and returned to the animal. 2-deoxy-D-[2,6-^{3}H]glucose and [U-^{14}C]glucose (PerkinElmer, Melbourne, Australia) were administered as an intravenous bolus in the last 30 min of the glucose infusion. Blood samples were taken 2, 5, 10, 15, 20 and 30min after administration of the tracer bolus for estimation of tracer clearance and blood glucose. Animals were then euthanized and tissues were rapidly removed, freeze-clamped, and stored at -80°C for later analysis. TC muscle was powdered prior to any assay procedure to make sure the tissue was in a homogenous state.

Analytic Methods

Blood and plasma glucose levels (YSI2300; Yellow Springs Instruments, Yellow Springs, OH, USA), and plasma insulin (Rat RIA, Millipore, Missouri, USA) were measured.

Plasma and tissue levels of ^{3}H- and ^{14}C-labelled tracers were measured to calculate whole body glucose disposal rate (R_d), to estimate tissue glucose uptake (R_g'), and to measure glucose incorporation rate into glycogen. Assays and calculations are as previously described [22]. Glycogen concentration was measured as previously described [12]

Oxidation of palmitate and glutamate was assessed in muscle tissue homogenates as described previously [19].

Immunoblotting

Protein Extraction: 24h post transfection, cells were washed once with PBS and collected in RIPA buffer (65 mM Tris (pH 7.4), 150 mM, NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10% glycerol, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 10mM sodium fluoride, 1 mM Na_3VO_4, 1 mM PMSF and 50mM nicotinamide) and snap frozen. On the first thaw, cells were sonicated and incubated for 1h. For the TC muscles approximately 50 mg of powdered TC was homogenised in RIPA buffer and incubated for 1-2h. Lysates from cells and tissues were centrifuged at 12,000 g to remove any insoluble particles and protein concentration was determined via a protein assay (BioRad).

Immunoblot analysis: Cell and tissue lysates were subjected to SDS-PAGE, transferred to PVDF membranes, blocked in 2-5% BSA and then immunoblotted with antibodies for SIRT1, Acetyl-p53 (K379), p53, insulin receptor, p-Akt (S473), Akt, pACC (S79), ACC,

VDAC/Porin (all from Cell Signaling), p-insulin receptor (Y1162/3; Invitrogen), OXPHOS (complexes I, II, III, V; MitoSciences), complex IV (Molecular Probes). Densitometry analysis was performed using ImageJ software (NIH; http://imagej.nih.gov/ij/).

Statistics

Data are expressed as means ± SEM. Differences between groups were determined by one-, or two-way ANOVA as appropriate. If the one-way ANOVA reached significance a Newman-Keuls multiple comparison post hoc test was conducted. If the two-way ANOVA reached significance a Bonferroni's post hoc test was conducted. All statistical analysis was performed using GraphPad Prism (Version 6 for Windows, GraphPad Software, San Diego, California, USA). Significance was set at $p \leq 0.05$.

Results

In Vitro

To confirm the activity of the constructs, C2C12 myoblasts were successfully transfected with SIRT1, SIRT1 (H363Y) or empty vector for 24h (Figure 1A). The tumour suppressor protein p53 is a well-described target of SIRT1 and as shown in Figure 1B, there was a decrease in acetyl-p53 in the SIRT1 overexpressing myoblasts when compared to the empty vector and no change with the mutated version. Thus the SIRT1 construct was active and the mutated version was not. These constructs were used to increase SIRT1 protein in skeletal muscle of rats.

In Vivo

Basal

Electroporation of the SIRT1 and SIRT1 (H363Y) constructs significantly increased the levels these proteins in the test leg (right) compared to the control leg (left; Figure 2A, 2B). To determine if overexpression of either of the SIRT1 constructs altered mitochondrial parameters we examined the content of the mitochondrial respiratory chain and porin, an abundant mitochondrial protein often used as a marker of mitochondrial density in control muscle and muscle overexprerssing the SIRT1 constructs. There was no difference in these parameters in the left and right tibialis muscles (Figure 2C and D). As a measure of function, whole tissue homogenate oxidation rates of palmitate and glutamate were measured. Palmitate oxidation showed a small (11%), but significant ($p=0.049$) increase in the SIRT1 overexpressing leg and no change in the SIRT1 (H363Y) overexpressing leg (Figure 2E). When glutamate was used no difference in substrate oxidation was observed in either the SIRT1 or SIRT1 (H363Y) overexpressing leg (Figure 2F). This data suggests mitochondrial

content and function were largely unaltered by the overexpression of either an active or inactive version of SIRT1 for 1 week.

Glucose Infusion

Blood glucose levels increased significantly in glucose-infused animals and remained stable over the 5h infusion period ($p<0.05$; Table 1). Plasma insulin was also elevated and remained stable during glucose infusion ($p<0.05$; Table 1). The amount of glucose infused to maintain glycemia decreased significantly between 3h and 5h of infusion, indicating the existence of significant whole body insulin resistance in 5h glucose-infused rats ($p<0.05$; Table 1).

After 5h glucose infusion, tibialis muscle glucose uptake was assessed as previously described [12-14]. Overexpression of SIRT1 or SIRT1 (H363Y) had no effect on glucose uptake into tibialis muscle (Figure 3A). There were also no differences between the test and control legs in glycogen synthesis or content after 5h infusion, irrespective of which construct was overexpressed (Figure 3B and C).

Immunobloting for phosphorylation status of insulin receptor (IR) or Akt showed neither construct altered basal phosphorylation status of these proteins nor the increase seen in response to the infusion (Figure 4A and B). In the current study, there was also no difference in the basal phosphorylation state of ACC or the decrease in phosphorylation of ACC in response to hyperglycaemia in the SIRT1, or SIRT1 (H363Y), over expressing leg (Figure 4C). This indicates that AMPK activity was unlikely to be affected by overexpression of SIRT1.

Discussion

The current study provides evidence that increasing SIRT1 has no obvious impact on the development of glucose-induced insulin resistance in skeletal muscle. When SIRT1 or the deacetylase inactive version of SIRT1 (H363Y) were over expressed in muscle, there was no improvement, nor worsening, of the insulin resistance that occurs with 5h of hyperglycaemia (Figure 3). In previous *in vitro* studies, an increase in SIRT1 protein or activity was associated with a rescuing of the effects of incubation with high glucose [9,10]. One possibility for the differences between the previous *in vitro* and current *in vivo* studies is that *in vivo* activity of SIRT1 is tightly regulated by intracellular NAD^+ content. Under such conditions it is possible that a decreased enzyme activity of SIRT1 cannot be overcome by simply overexpressing the protein. Other possibilities for the apparently different effects of SIRT1 overexpression are the origin of the cells used for in vitro studies (mesangial cells [9] or endothelial cells [8,10]), *in vivo* vs *in vitro* conditions, and/or duration of glucose exposure. Most studies, especially those in cells, use at least 24h of the hyperglycaemic insult, whereas our study examined 5h glucose infusion.

It has been reported previously that SIRT1 can modulate the insulin signaling pathway via repression of PTP1B [4], Rictor of the mTORC2 complex [23], or via regulation of the p85 [24], p55α and p50α [25] subunits of PI3K. In the current study we did not find any differences in the phosphorylation status of the insulin receptor, a target of PTP1B, or Akt, which is downstream of both mTORC2 and PI3K, either in the basal state or in response to hyperglycaemia (Figure 4A and B). This indicates that one week of overexpression of SIRT1 protein is insufficient to modulate these indicators of insulin signaling pathway activity although this does not preclude that longer term increases in SIRT1 protein may have some effects.

Similarly, SIRT1 has been reported to regulate AMPK signaling through deacetylation and activation of its upstream kinase LKB1 [26]. Conversely, AMPK is thought to regulate SIRT1 activity by modulating NAD^+ levels [27]. However phosphorylation of ACC, a well described target of AMPK, was not changed by overexpression of SIRT1 in either the basal state or in response to hyperglycaemia (Figure 4C) suggesting unaltered AMPK signaling in this model. This lack of effect of SIRT1 on AMPK and ACC is consistent with other studies reporting no difference in the phosphorylation state or activity of AMPK and ACC when SIRT1 is specifically knocked out [25,28], or overexpressed [29] in muscle.

Although there is evidence for a role of SIRT1 in mitochondrial biogenesis, we show that there was also no change in markers of mitochondrial content (Figure 2A and B), or function (Figure 2C and D), in muscle after overexpression of SIRT1 for one week. Recently however, there has been some controversy about the role SIRT1 plays in mitochondrial biogenesis in muscle, especially *in vivo*. Supporting its role, a comprehensive study in C2C12 muscle cells showed that SIRT1, through its ability to deacetylate and activate PGC-1α, was intimately linked to mitochondrial biogenesis and function [30]. *In vivo*, studies in conditional whole body knockout [31], muscle specific knockout [32] as well as whole body over expression [31] of SIRT1 show similar links. Other studies by Philp *et al* [28] reported that muscle specific knockout of SIRT1 does not alter the abundance or activities of complexes within the respiratory chain, or mitochondrial content. Interestingly though, when SIRT1 was overexpressed using a similar electroporation protocol to ours, there was a decrease in components of the respiratory chain [33,34] while a dominant negative version of SIRT1 (H355Y) of SIRT1 increased these components [34]. However, these studies did harvest tissues after 14 days which could influence the impact of increasing SIRT1 on mitochondrial

capacity in muscle. The role of SIRT1 in regulating muscle mitochondrial function under normal conditions as well as in response to exercise or resveratrol treatment will remain controversial until the results of more studies using similar paradigms become available [35-37]

In conclusion, our results suggest that it is unlikely that changes in SIRT1 are involved in the development of insulin resistance in skeletal muscle in response to hyperglycaemia. This data also adds to the controversy surrounding the exact role of SIRT1 in muscle mitochondrial biogenesis *in vivo*. The acetylation/sirtuin axis is clearly complex and changes in SIRT1 protein may not equate to linear changes in enzyme activity, due to requirement of NAD^+ as a co-factor. Therefore strategies aimed at raising NAD^+ might be the way forward in examining the role of SIRT1 in the acute regulation of muscle metabolism.

Acknowledgments

GJC and EWK are supported by the National Health and Medical Research Council (NHMRC) of Australia's Fellowship Scheme. NT is an Australian Research Council (ARC) Future Fellow. This study was also funded by a grant to NBR and EWK from the National Institutes of Health, USA (RO1DK067509).

The Authors have no conflicts of interest to declare.

References

1. Nogueiras R, Habegger KM, Chaudhary N, Finan B, Banks AS, et al. (2012) Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. Physiol Rev 92: 1479-1514.
2. Bordone L, Cohen D, Robinson A, Motta MC, van Veen E, et al. (2007) SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell 6: 759-767.
3. Boily G, Seifert EL, Bevilacqua L, He XH, Sabourin G, et al. (2008) SirT1 regulates energy metabolism and response to caloric restriction in mice. PLoS One 3: e1759.
4. Sun C, Zhang F, Ge X, Yan T, Chen X, et al. (2007) SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab 6: 307-319.
5. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, et al. (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 434: 113-118.
6. Suchankova G, Nelson LE, Gerhart-Hines Z, Kelly M, Gauthier MS, et al. (2009) Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. Biochem Biophys Res Commun 378: 836-841.
7. Balestrieri ML, Rienzo M, Felice F, Rossiello R, Grimaldi V, et al. (2008) High glucose downregulates endothelial progenitor cell number via SIRT1. Biochim Biophys Acta 1784: 936-945.
8. Yuan Q, Chen L, Xiang DX, Li YJ, Hu CP (2011) Effect of resveratrol derivative BTM-0512 on high glucose-induced dysfunction of endothelial cells: role of SIRT1. Can J Physiol Pharmacol 89: 713-722.
9. Xu Y, Nie L, Yin YG, Tang JL, Zhou JY, et al. (2012) Resveratrol protects against hyperglycemia-induced oxidative damage to mitochondria by activating SIRT1 in rat mesangial cells. Toxicol Appl Pharmacol 259: 395-401.
10. Wang Y, Zhao X, Shi D, Chen P, Yu Y, et al. (2013) Overexpression of SIRT1 Promotes High Glucose-Attenuated Corneal Epithelial Wound Healing via p53 Regulation of the IGFBP3/IGF-1R/AKT Pathway. Invest Ophthalmol Vis Sci 54: 3806-3814.
11. Nedachi T, Kadotani A, Ariga M, Katagiri H, Kanzaki M (2008) Ambient glucose levels qualify the potency of insulin myogenic actions by regulating SIRT1 and FoxO3a in C2C12 myocytes. Am J Physiol Endocrinol Metab 294: E668-678.
12. Brandon AE, Hoy AJ, Wright LE, Turner N, Hegarty BD, et al. (2011) The evolution of insulin resistance in muscle of the glucose infused rat. Arch Biochem Biophys 509: 133-141.
13. Hoy AJ, Bruce CR, Cederberg A, Turner N, James DE, et al. (2007) Glucose infusion causes insulin resistance in skeletal muscle of rats without changes in Akt and AS160 phosphorylation. Am J Physiol Endocrinol Metab 293: E1358-1364.
14. Kraegen EW, Saha AK, Preston E, Wilks D, Hoy AJ, et al. (2006) Increased malonyl-CoA and diacylglycerol content and reduced AMPK activity accompany insulin resistance induced by glucose infusion in muscle and liver of rats. American Journal of Physiology - Endocrinology & Metabolism 290: E471-479.
15. Saha AK, Xu XJ, Lawson E, Deoliveira R, Brandon AE, et al. (2010) Downregulation of AMPK accompanies leucine- and glucose-induced increases in protein synthesis and insulin resistance in rat skeletal muscle. Diabetes 59: 2426-2434.
16. Zu Y, Liu L, Lee MY, Xu C, Liang Y, et al. (2010) SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells. Circ Res 106: 1384-1393.
17. Bruce CR, Brolin C, Turner N, Cleasby ME, van der Leij FR, et al. (2007) Overexpression of carnitine palmitoyltransferase I in skeletal muscle in vivo increases

fatty acid oxidation and reduces triacylglycerol esterification. American Journal of Physiology - Endocrinology & Metabolism 292: E1231-1237.
18. Cleasby ME, Davey JR, Reinten TA, Graham MW, James DE, et al. (2005) Acute bidirectional manipulation of muscle glucose uptake by in vivo electrotransfer of constructs targeting glucose transporter genes. Diabetes 54: 2702-2711.
19. Wright LE, Brandon AE, Hoy AJ, Forsberg GB, Lelliott CJ, et al. (2011) Amelioration of lipid-induced insulin resistance in rat skeletal muscle by overexpression of Pgc-1beta involves reductions in long-chain acyl-CoA levels and oxidative stress. Diabetologia.
20. McMahon JM, Signori E, Wells KE, Fazio VM, Wells DJ (2001) Optimisation of electrotransfer of plasmid into skeletal muscle by pretreatment with hyaluronidase -- increased expression with reduced muscle damage. Gene Ther 8: 1264-1270.
21. Hoy AJ, Brandon AE, Turner N, Watt MJ, Bruce CR, et al. (2009) Lipid and insulin infusion-induced skeletal muscle insulin resistance is likely due to metabolic feedback and not changes in IRS-1, Akt or AS160 phosphorylation. Am J Physiol Endocrinol Metab: E67-75.
22. James DE, Jenkins AB, Kraegen EW (1985) Heterogeneity of insulin action in individual muscles in vivo: euglycemic clamp studies in rats. American Journal of Physiology 248: E567-574.
23. Wang RH, Kim HS, Xiao C, Xu X, Gavrilova O, et al. (2011) Hepatic Sirt1 deficiency in mice impairs mTorc2/Akt signaling and results in hyperglycemia, oxidative damage, and insulin resistance. J Clin Invest 121: 4477-4490.
24. Frojdo S, Durand C, Molin L, Carey AL, El-Osta A, et al. (2011) Phosphoinositide 3-kinase as a novel functional target for the regulation of the insulin signaling pathway by SIRT1. Mol Cell Endocrinol 335: 166-176.
25. Schenk S, McCurdy CE, Philp A, Chen MZ, Holliday MJ, et al. (2011) Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. J Clin Invest 121: 4281-4288.
26. Lan F, Cacicedo JM, Ruderman N, Ido Y (2008) SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. J Biol Chem 283: 27628-27635.
27. Canto C, Auwerx J (2009) PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 20: 98-105.
28. Philp A, Chen A, Lan D, Meyer GA, Murphy AN, et al. (2011) Sirtuin 1 (SIRT1) deacetylase activity is not required for mitochondrial biogenesis or peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) deacetylation following endurance exercise. J Biol Chem 286: 30561-30570.
29. White AT, McCurdy CE, Philp A, Hamilton DL, Johnson CD, et al. (2013) Skeletal muscle-specific overexpression of SIRT1 does not enhance whole-body energy expenditure or insulin sensitivity in young mice. Diabetologia 56: 1629-1637.
30. Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, et al. (2007) Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. Embo J 26: 1913-1923.
31. Price NL, Gomes AP, Ling AJ, Duarte FV, Martin-Montalvo A, et al. (2012) SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab 15: 675-690.
32. Menzies KJ, Singh K, Saleem A, Hood DA (2013) Sirtuin 1-mediated effects of exercise and resveratrol on mitochondrial biogenesis. J Biol Chem 288: 6968-6979.
33. Gurd BJ, Yoshida Y, Lally J, Holloway GP, Bonen A (2009) The deacetylase enzyme SIRT1 is not associated with oxidative capacity in rat heart and skeletal muscle and its overexpression reduces mitochondrial biogenesis. J Physiol 587: 1817-1828.

34. Higashida K, Kim SH, Jung SR, Asaka M, Holloszy JO, et al. (2013) Effects of Resveratrol and SIRT1 on PGC-1alpha Activity and Mitochondrial Biogenesis: A Reevaluation. PLoS Biol 11: e1001603.
35. Menzies KJ, Chabi B, Hood DA, Schenk S, Philp A, et al. (2012) Commentaries on viewpoint: does SIRT1 determine exercise-induced skeletal muscle mitochondrial biogenesis: differences between in vitro and in vivo experiments? J Appl Physiol (1985) 112: 929-930.
36. Gurd BJ, Little JP, Perry CG (2012) Does SIRT1 determine exercise-induced skeletal muscle mitochondrial biogenesis: differences between in vitro and in vivo experiments? J Appl Physiol (1985) 112: 926-928.
37. Gurd BJ, Little JP, Perry CG (2012) Last word on viewpoint: does SIRT1 determine exercise-induced skeletal muscle mitochondrial biogenesis: differences between in vitro and in vivo experiments? J Appl Physiol (1985) 112: 931.

Tables

Table 1: Blood, plasma and whole body parameters of basal and glucose infused animals.

	Basal	1h	3h	5h
Blood Glucose (mM)	4.5 ± 0.1	9.7 ± 0.5*	13.2 ± 0.4*	12.4 ± 0.4*
Plasma Insulin (mU/L)	40 ± 4	236 ± 23*	309 ± 24*	337 ± 21*
Glucose Infusion rate (mg/kg/min)	-	69.4 ± 1.8	70.0 ± 2.0	55.5 ± 1.5‡

Data are expressed as means ± SEM. *$p<0.05$ compared to basal. ‡$p<0.05$ compared to 1h and 3h.

Figures

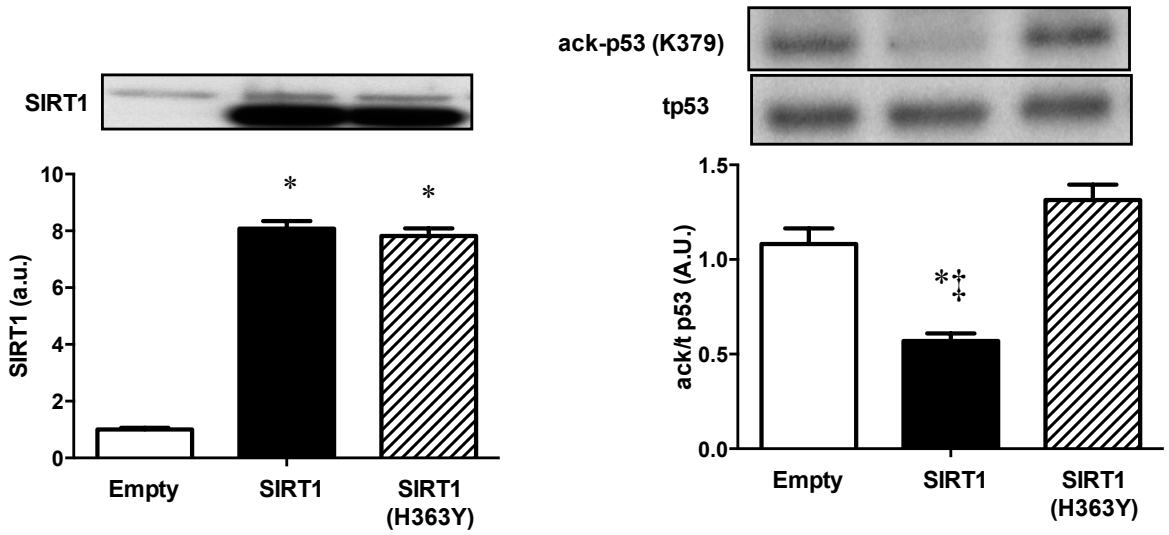

Figure 1: Effect of SIRT1 or SIRT1 (H363Y) overexpression on acetylation of p53 in C2C12 myoblasts. (A) Protein expression of SIRT1 or SIRT1 (H363Y) in C2C12 myoblasts; (B) acetyl-p53 (K376) in C2C12 myoblasts overexpressing empty vector, SIRT1 or SIRT1 (H363Y). *$p<0.01$ vs empty vector; ‡ $p<0.01$ vs SIRT1 (H363Y). Data are expressed as means ± SEM.

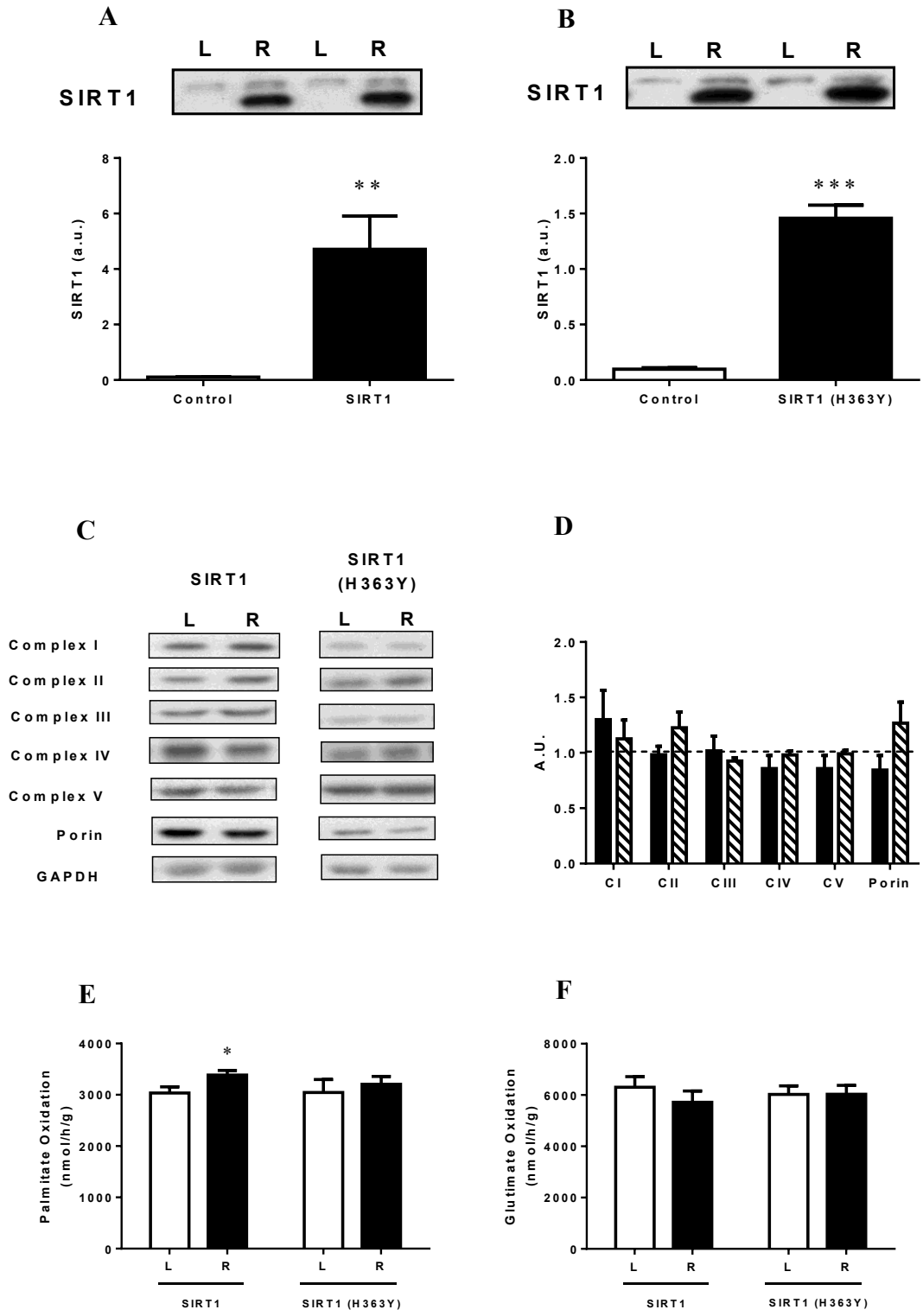

Figure 2: Effect of SIRT1 and SIRT1 (H363Y) overexpression on mitochondrial proteins and function in the basal state. (A) SIRT1 and (B) SIRT1 (H363Y) over expression in TC muscle. (C) Representative immunoblots for mitochondrial proteins and (D) densotrometric quantitation of these protein blots expressed as relative to the control leg (dotted line; Black bars SIRT1; Hatched bars SIRT1 (H363Y)). (E) oxidation rates of muscle homogenates incubated in medium containing palmitate or (F) glutamate; * p=0.049; **p<0.01; ***p<0.001 vs control leg. Data are expressed as means ± SEM.