Neuronal Sirt1 Deficiency Increases Insulin Sensitivity in Both Brain and Peripheral Tissues

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Background:
Sirt1 is a NAD+ dependent class III deacetylase and a cellular energy sensor.

Results:
Selective removal of Sirt1 in neurons causes increased central and systemic insulin sensitivity.

Conclusion:
Neuronal Sirt1 plays a physiological role to inhibit both hypothalamic insulin sensitivity and whole body metabolism.

Significance:
CNS Sirt1 inhibition is protective against metabolic diseases and hence non-braining-penetrating Sirt1 activators may have greatest therapeutic potential.
Summary
Sirt1 is a NAD+ dependent class III deacetylase that functions as a cellular energy sensor. In addition to its well-characterized effects in peripheral tissues, emerging evidence suggests that neuronal Sirt1 activity plays a role in the central regulation of energy balance and glucose metabolism. To assess this idea, we generated Sirt1 neuron-specific knockout (SINKO) mice. On both standard chow and HFD, SINKO mice were more insulin sensitive than Sirt1+/- mice. Thus, SINKO mice had lower fasting insulin levels, improved glucose tolerance and insulin tolerance, and enhanced systemic insulin sensitivity during hyperinsulinemic euglycemic clamp studies. Hypothalamic insulin sensitivity of SINKO mice was also increased over controls, as assessed by hypothalamic activation of PI3K, phosphorylation of Akt and FoxO1 following systemic insulin injection. Intracerebroventricular injection of insulin led to a greater systemic effect to improve glucose tolerance and insulin sensitivity in SINKO mice compared to controls. In line with the in vivo results, insulin-induced AKT and FoxO1 phosphorylation were potentiated by inhibition of Sirt1 in a cultured hypothalamic cell line. Mechanistically, this effect was traced to a reduced effect of Sirt1 to directly deacetylate and repress IRS-1 function. The enhanced central insulin signaling in SINKO mice was accompanied by increased insulin receptor signal transduction in liver, muscle and adipose tissue. In summary, we conclude that neuronal Sirt1 negatively regulates hypothalamic insulin signaling, leading to systemic insulin resistance. Interventions that reduce neuronal Sirt1 activity have the potential to improve systemic insulin action and limit weight gain on an obesigenic diet.

Introduction:
Insulin resistance is a defining pathophysiologic feature of Type 2 diabetes mellitus, and obesity is the most common cause of insulin resistance in the United States and other Western countries(1). Insulin resistance is characterized by an impaired ability of insulin to enhance muscle glucose uptake, suppress adipose tissue lipolysis, and suppress hepatic glucose production(1). Studies have shown that the central nervous system (CNS) can influence systemic insulin action and glucose homeostasis in part by regulating feeding behavior and energy expenditure in response to various nutrients and hormones(2). In addition, insulin inhibits hepatic glucose output via both direct and indirect pathways, the latter relying on a CNS neuronal network(3). Furthermore, Scherer et al. demonstrated that even a minimal amount of insulin administered into the CNS inhibited lipolysis in adipose tissue(4). These findings highlight a potentially important role for neuronal insulin signaling in whole body insulin action.

Sirtuin 1 (Sirt1), a member of the sirtuin protein family, is a class III histone deacetylase(5). The yeast homolog of Sirt1, Silent Information Regulator 2 (Sir2), is implicated in stress responses and the increased lifespan caused by calorie restriction. The enzymatic activity of sirtuins requires NAD+, suggesting these enzymes function as cellular energy sensors, with enzyme activity typically increasing during periods of negative energy balance. Indeed, Sirt1 activation during fasting influences peripheral metabolic responses including fatty acid oxidation, insulin secretion, insulin action, adipogenesis, gluconeogenesis, and inflammation(5).

While a consensus has formed surrounding the beneficial effects of Sirt1 in peripheral tissues, evidence of a physiological role for Sirt1 in the CNS is mixed and somewhat controversial. For example, SIRT1 expression levels increase in the hypothalamus with fasting and regulate food intake.(6) On a mixed genetic background, Ramadori, et al., reported that selective knockout (KO) of Sirt1 in pro-opiomelanocortin (POMC) neurons, a key hypothalamic neuronal subset for energy homeostasis, decreases energy expenditure and increase susceptibility to diet-induced obesity (DIO) in mice(7). In contrast, both intracerebroventricular (i.c.v.) injection of EX-527, a specific Sirt1 inhibitor, and deletion of Sirt1 in Agouti-related peptide (AgRP) neurons (situated adjacent to POMC cells in the hypothalamic arcuate nucleus (ARC), cause decreases of food intake and body weight in mice and rats(8,9). Thus, it appears that Sirt1 in different neuronal populations may play distinctive and sometimes even opposing roles in energy balance regulation. With respect to insulin sensitivity, whether Sirt1 in CNS directly influences central and/or peripheral insulin sensitivity is also controversial. In light of these results, we sought to ascertain the role of global neuronal Sirt1 in animals on a pure genetic background and without
developmental problems that have confounded other analyses(10,11) so as to gain an improved understanding of the biological function of neuronal Sirt1.

In the current work, we studied the physiological role of neuronal Sirt1 with respect to changes in insulin sensitivity associated with aging, high-fat (HF) feeding, and caloric restriction by generating and characterizing neuron-specific Sirt1 knockout (Neuronal KO; SINKO) mice. We show for the first time that on both standard chow and HF diet, selective removal of Sirt1 in neurons causes increased systemic insulin sensitivity. SINKO mice remained insulin sensitive during aging and showed greater metabolic improvement than control mice during chronic calorie restriction. The improved systemic insulin sensitivity of SINKO mice was associated with improved CNS insulin signaling in the hypothalamus. These findings indicate that neuronal Sirt1 plays a physiological role to inhibit both hypothalamic insulin sensitivity and whole body glucose homeostasis.

Results:

Sirt1 Neuron–Specific Knockout Mice (SINKO mice)

To investigate the function of neuronal Sirt1, we generated SINKO mice using the synapsin 1 Cre−LoxP system. Cre expression driven by the Synapsin 1 promoter (Syn−Cre mice) leads to recombination in neurons, but not other cell types in the brain(6,12). The deletion of the floxed exon4 of the Sirt1 gene, which encodes the deacetylase domain, does not cause a frame shift of Sirt1 protein translation(11). Therefore, Cre expression results in a mutant Sirt1 protein with deacetylase deficiency but an intact C-terminus. To verify brain–specific deletion of Sirt1 in SINKO mice, we isolated RNA from multiple brain regions and from peripheral tissues. Quantitative PCR (qPCR) for measurement of Sirt1 exon4 showed that brain Sirt1 deletion was regional, with markedly reduced mRNA abundance in spinal cord, brain stem, hypothalamus, diencephalon and hippocampus, mildly reduced expression in cerebral cortex and cerebellum, and unchanged levels in olfactory bulb and pituitary, compared to Sirt1ff mice (Fig. 1A). Tissues other than brain displayed normal Sirt1 expression (Fig. 1B). That the decrease of Sirt1 mRNA was not more pronounced in SINKO vs. control mice (Fig. 1A) likely reflects the neuron-specific nature of our gene targeting, with Sirt1 expression being unaffected in glial, endothelial and other brain cell types. Interestingly, the deletion of exon4 in SINKO hypothalamus has not only resulted in a molecular weight shift of the mutant protein, but also caused a reduction of total Sirt1 protein content (Fig. 1C), a phenomenon that has been reported previously in other Sirt1 KO models(13-15). To further provide evidence for the magnitude of the Sirt1 deacetylase deletion, we found a marked increase in acetylation of the well-known Sirt1 targets p53 and Stat3 in hypothalamic extracts of SINKO mice (Fig. 1C). However, we did not detect hyperacetylation of PGC1α or FoxO1 (Supplemental fig. 1A). PCR detection of genomic DNA (data not shown) and RNA (Fig. 1D) from various tissues demonstrated Cre–mediated recombination in brain, but not in gut, muscle, liver, adipose tissue, spleen, and pancreatic islets (Fig. 1D). Interestingly, Cre-mediated recombination was not detected in trigeminal nerve ganglion cells (Fig. 1D), suggesting that Sirt1 deletion was limited only to the CNS neurons, and did not occur in peripheral neurons. This KO expression pattern is comparable to what we have reported for Syn-Cre driven PPARγ deletion(12).

Glucose and Insulin Homeostasis

On a standard chow diet, SINKO mice displayed slightly lower body weight (Fig. 2A), while body composition analysis showed no decrease in fat mass (Fig. 2B). Growth and fertility were normal in SINKO mice, and there were no differences in brain or pituitary size, serum growth hormone levels, hepatic IGF1 gene expression, or body length (Supplemental figure 1B-F) between control and SINKO mice. Mice of both genotypes consumed similar amounts of food on a chow diet (Supplemental figure 1G). Since Sirt1ff mice exhibited the same glucose tolerance and fasting insulin level as wild type (WT) mice on both standard chow and HFD (Supplemental figure 1H and 1I), we chose to use Sirt1ff mice as controls throughout our study. To determine whether neuronal Sirt1 plays a physiological role to regulate insulin sensitivity, we first performed oral glucose tolerance tests (OGTTs) on chow-fed Sirt1ff and SINKO mice. Although the plasma glucose excursion was comparable in the two groups (Fig. 2C), plasma insulin levels were markedly reduced
in SINKO mice (Fig. 2D), consistent with increased insulin sensitivity. This finding was corroborated by demonstrating an enhanced ability of insulin to lower blood glucose during insulin tolerance testing (ITT) in SINKO mice (supplemental figure 2A). To investigate whether either insulin resistance that accompanies aging or the response to caloric restriction (CR) were altered in SINKO mice, we repeated GTTs in 16-month old mice that were either fed ad libitum or provided with only 60% of estimated daily calories. Since it is known that caloric restriction significantly improves glucose metabolism, a higher dose of glucose was used for these OGTTs (2 g/kg. Compared to 6 month-old controls, fasting insulin levels were increased in 16-month old mice that were fed ad libitum (Fig. 2E), consistent with aging-induced insulin resistance. This effect of aging was attenuated by caloric restriction, which normalized serum insulin levels and improved glucose tolerance in 16-month old control mice, and this beneficial effect was substantially enhanced in SINKO mice. Notably, under each of these circumstances SINKO mice displayed improved metabolic profiles compared to Sirt1f/f controls, as indicated by lower basal serum insulin levels (Fig. 2E), lower glucose excursion upon CR (Fig. 2F), and lower glucose-induced insulin secretion (Fig. 2G). In summary, SINKO mice display increased insulin sensitivity, are protected from aging-induced insulin resistance and respond better than control mice to CR.

### Quantitation of In Vivo and In Vitro Insulin Sensitivity

We next investigated whether neuron-specific Sirt1 deletion attenuates the deleterious metabolic effects of HF feeding. On a HFD, SINKO mice gained somewhat less weight than controls, amounting to an ~4 gm difference at 16 wks (Fig. 2A), and body composition results showed a corresponding but very modest decrease of total fat mass in these animals (Fig. 2B). Since changes in body weight/fat mass can influence metabolic homeostasis, we sought to distinguish between effects of Sirt1 deletion versus effects of body weight on glucose homeostasis and insulin sensitivity. Since individual body weights overlapped between genotypes, we used control and SINKO animals that were matched for both body weight and body composition (see Methods) in our metabolic studies. We found that after consuming HF diet for 12 wk, a more robust phenotype emerged compared to the chow-fed studies, as SINKO mice were substantially more glucose tolerant than controls (Fig. 3A), with reduced fasting and post-glucose challenge insulin levels (Fig. 3B), suggestive of improved insulin sensitivity. This idea was strengthened by the ITT results, which revealed a greater glucose-lowering effect of insulin in SINKO mice. Notably, each of these in vivo measurements of insulin sensitivity were equally improved in SINKO mice when results from body weight and body composition-matched mice from the two genotypes were compared (Supplemental figure 3B-E), indicating a body weight-independent phenotype.

Hyperinsulinemic–euglycemic clamp studies were performed in weight- and body composition-matched control and SINKO mice (43.1 g ±1.6 v.s. 42.0 g ±1.3; Fig. 2D inset) maintained on either standard chow or HFD, to quantitate differences in insulin sensitivity and the contribution made by liver, muscle and adipose tissue to the phenotype. As seen in Fig. 3, both the glucose infusion rate (GIR) (Fig. 3D) and insulin-stimulated glucose disposal rate (IS-GDR) (Fig. 3E) were higher in lean chow-fed SINKO mice than in controls. More interestingly, GIR, IS-GDR, and the suppression of hepatic glucose production (HGP), were each greater in obese HFD SINKO mice than in weight-matched, HF-fed Sirt1f/f controls (Fig. 3F and 3G). Thus, neuron-specific deletion of Sirt1 attenuates insulin resistance in both muscle and liver during HF feeding. Moreover, basal HGP was reduced in HF-fed SINKO mice compared to HFD Sirt1f/f mice (Fig. 3F). In concert with the clamp studies, biochemical measures in tissues showed increased insulin-stimulated AKT phosphorylation in muscle, liver, and adipose tissue of SINKO mice (Fig. 3H-3J) without changes in total AKT levels or hepatic AMPK or CREB phosphorylation. Taken together, our results show that during HF feeding, SINKO mice are more insulin sensitive than Sirt1f/f mice, regardless of body weight.

### Adipose Biology and Inflammation

Noticeably, while HFD-induced weight gain was attenuated in Sirt1 SINKO mice relative to controls after 16 weeks, epididymal white adipose tissue (eWAT) mass was increased in SINKO mice and liver weight was decreased (Fig. 4A and 4B). Despite increased eWAT size in SINKO mice, there was no difference in adipocyte cell size (data not
shown). To test whether increased lipogenesis contributes to increased eWAT mass in SINKO mice, we measured adipose tissue lipid content and fatty acid composition. We found that eWAT diacylglyceride (DAG) content was reduced, whereas tissue levels of triglyceride (TAG) and fatty acids 14:0, 16:0, and 16:1n7 were increased in SINKO mice (Fig. 4D). As these fatty acids, particularly 16:1n7, are markers of de novo lipogenesis(16,17), our findings are consistent with the hypothesis that reduced neuronal Sirt1 signaling either directly or indirectly exerts a stimulatory effect on lipogenesis in eWAT. We also assessed adipose lipolysis by measuring serum glycerol and free fatty acid levels, but found no significant differences between genotypes (Supplemental figure 3A and 3B).

Interestingly, the elevated neutral lipid synthesis in SINKO adipose were not correlated with mRNA levels of sterol regulatory element binding protein 1c (SREBP1c), fatty acid synthase (FAS), or acetyl CoA carboxylase (ACC) (data not shown), and were rather associated with reduced adipocyte protein 2 (aP2) and increased stearoyl-Coenzyme A desaturase 1 (SCD-1) (Fig. 4E). Consuming a HFD is well known to induce adipose tissue inflammation, and this effect was suppressed in SINKO mice, as indicated by a marked reduction in macrophage infiltration (Fig. 4C), lower expression levels of pro-inflammatory genes (Fig. 4E), and reduced serum TNFα and IL6 levels (Fig. 4F). These changes were not associated with changes of circulating adiponectin or leptin levels (Supplemental figure 3C and 3D). In these groups, SINKO mice weighed less on average than controls (3~4 g). However these small differences do not produce noticeable changes in adipose tissue inflammation or lipid profiles in either genotypes. Furthermore, individual analyses revealed that weight-matched control and SINKO mice possessed the same phenotypic differences in the measurements (data not shown).

**Hepatic Lipids and Inflammation**

HFD markedly increases hepatic lipid content(18) and such changes were comparable in control and SINKO mice. Specifically, TAG, DAG, 14:0, 16:0, and 16:1n7 levels were the same in liver from control vs. SINKO mice (Fig. 4G and Supplemental figure. 3E). There was a trend (p=0.09) for decreased ceramide levels in SINKO livers (Fig. 4G). In addition, we found no differences in the fatty acid composition of TAGs and DAGs in liver between the genotypes (Supplemental Tables 1 and 2). Markers of liver tissue inflammation were also no different between genotypes (data not shown), although levels of mRNA encoding SOCS3 (suppressor of cytokine signaling-3), a negative regulator of insulin signaling, were down-regulated in SINKO mouse liver (Fig. 4H), consistent with our in vivo results showing increased adipose tissue and hepatic insulin sensitivity in SINKO mice.

**Effect of Neuronal Sirt1 Deletion on CNS Insulin Action**

Insulin action in the CNS regulates energy homeostasis and adipose tissue physiology(4,19), and CNS insulin resistance caused by inflammation or nutrient overload can lead to peripheral insulin resistance(19). One potential mechanism to explain increased insulin sensitivity in SINKO mice, therefore, is that neuronal insulin action is enhanced by Sirt1 deficiency. To test this hypothesis, we measured the ability of systemic insulin administration to activate the insulin signaling pathway in the hypothalamus of weight-matched, chow-fed control and SINKO mice. Intravenous insulin injection led to a greater stimulation of the insulin signaling pathway, as assessed by increased IRS1 tyrosine phosphorylation, IRS1-associated PI3K activity, and serine/threonine phosphorylation of AKT, FoxO1 and PKC, in the hypothalamus of SINKO vs. control mice (Fig. 5A and Fig. 5B).

Moreover, basal FoxO1 phosphorylation was also higher in SINKO hypothalamus (Fig. 5A). To directly test the systemic physiologic consequences of this increase in hypothalamic insulin sensitivity in SINKO mice, we conducted intracerebroventricular (i.c.v.) injection experiments. Following i.c.v insulin injection, glucose levels during GTTs were similar among the groups. However, insulin levels were reduced in the control mice, and were markedly lower in the SINKO mice (Fig. 5C), consistent with enhanced systemic insulin sensitivity following central insulin administration. More importantly, ITT studies showed that i.c.v vehicle injected SINKO mice were more insulin sensitive than vehicle injected control mice, and that i.c.v insulin injection had a much greater effect to enhance systemic insulin sensitivity in SINKO mice, compared to control mice (Fig. 5D). Together, these results argue that the enhanced central insulin sensitivity mediates the
whole body insulin sensitive phenotype in SINKO mice. To investigate this hypothesis further, we conducted studies with the hypothalamic neuronal cell line GT1-7. Adenovirus-mediated overexpression of a dominant-negative Sirt1 (H363Y; Sirt1-DN)(20) and siRNA-mediated sirt1 knockdown increased overall lysine acetylation (Supplemental figure 4). Insulin treatment induced phosphorylation of IRS-1, AKT, and FoxO1 (Fig. 6A), as expected, and this effect was potentiated by either dominant-negative Sirt1 or Sirt1 knockdown (Fig. 6A and 6B). These in vitro results offer direct evidence that in hypothalamic neurons, Sirt1 exerts a cell autonomous inhibitory effect on insulin signal transduction, as suggested by our in vivo finding of enhanced hypothalamic insulin signaling in insulin-treated SINKO mice (Fig. 5A).

Previous studies indicate that under basal conditions, IRS-1 is acetylated and that this acetylation is permissive for insulin signaling(21). Since tyrosine phosphorylation of IRS1 was enhanced by Sirt1 deficiency both in vitro and in vivo (Fig. 5A and 6B), we hypothesized that IRS-1 might be a relevant deacetylation target of Sirt1 in neurons. Indeed, immunoprecipitation experiments showed that endogenous IRS-1 physically interacted with Sirt1 in GT1-7 cells (Fig. 6C). Furthermore, IRS-1 acetylation was enhanced by Sirt1 inhibition (Fig. 6D). Taken together, these experiments suggest that inhibition of Sirt1 enhances neuronal insulin sensitivity by increasing IRS-1 acetylation.

**Energy Homeostasis:**

We next evaluated how neuronal Sirt1 influences energy homeostasis and ambulation. Under chow-fed conditions, 24-h ambulatory activity, energy intake, and energy expenditure were similar between control and SINKO mice (Supplemental Fig. 5A-B). Whereas food consumption in SINKO mice was comparable to controls during HF feeding (Supplemental Fig. 5C), energy expenditure tended to be increased in these mice via a mechanism that was independent of body weight, activity, and fat mass. Although this difference of energy expenditure did not achieve statistical significance (p = 0.066; Supplemental Figure 5D), even a very modest increase of EE can reduce weight gain during HF feeding, provided there is no difference in energy intake between groups (Supplemental Table 3).

**Discussion:**

The current studies investigated the role of neuronal Sirt1 in energy homeostasis and glucose metabolism by characterizing the phenotype of mice with neuron-specific Sirt1 knockout. We report that on both standard chow and a HF diet, deletion of Sirt1 in neurons increased systemic insulin sensitivity, an effect accompanied by increased central insulin signaling in the hypothalamus. These observations indicate that Sirt1 tonically inhibits neuronal insulin signaling, and support a model in which the effect of Sirt1 deletion to enhance hypothalamic insulin action mediates the improvement in systemic insulin sensitivity. Enhanced insulin sensitivity in these mice was also observed both during aging and in response to calorie restriction. Since SINKO mice exhibited modest attenuation of weight gain during HF feeding compared to littermate controls, likely involving an increase of EE with normal food intake, the possibility must be considered that their increased insulin sensitivity was secondary to reduced obesity. To control for this possibility, we conducted our metabolic studies in groups of experimental and control mice that were matched for body weight and body composition. Consequently, the enhanced insulin sensitivity we observed in SINKO mice cannot be attributed to differences in body weight, and must therefore arise directly from reduced neuronal Sirt1 signaling.

A central question posed by our studies is: how does neuronal Sirt1 deletion increase peripheral tissue insulin sensitivity? The possibility that increased hypothalamic insulin signaling mediates this effect is supported by evidence that hypothalami of SINKO mice displayed increased insulin signaling in response to peripheral insulin administration, including increased IRS1 tyrosine phosphorylation, increased IRS1-associated PI3K activity, as well as increased serine/threonine phosphorylation of Akt, FoxO1, and PKC. In direct experiments to support this concept, i.c.v insulin injection studies showed that central insulin stimulation had a much greater effect to improve peripheral insulin sensitivity in SINKO mice compared to controls. These results argue strongly that the effect of neuronal Sirt1 deletion to enhance central (hypothalamic) insulin sensitivity is the
mechanism underlying the improved systemic insulin sensitivity. Our cell culture studies showing that Sirt1 deficiency potentiates insulin-stimulated phosphorylation of Akt and FoxO1 in GT1-7 hypothalamic cells suggest a cell-autonomous effect of Sirt1-mediated deacetylation to blunt neuronal insulin action. Since this improvement of neuronal insulin action was not accompanied by altered FoxO1 deacetylation, we reasoned that Sirt1 acts upstream of FoxO1 by interfering with the insulin signaling cascade. In support of this hypothesis, we found that Sirt1 inhibition in cultured neurons caused hyper-acetylation of IRS-1, a protein known to have multiple acetylated lysine residues based on large-scale proteomic studies(22). Interestingly, Kaiser, et al., reported that IRS1 acetylation enhances insulin action by promoting insulin-induced IRS-1 tyrosine phosphorylation of the protein(21). This finding is consistent with a model in which IRS1 hyper-acetylation mediates increased hypothalamic insulin sensitivity arising from neuronal deletion of Sirt1. Furthermore, this effect appears to be relatively specific for IRS1, since we detected no changes in acetylation status of other known targets of Sirt1 in cultured hypothalamic cells. Clearly, further work is necessary to map the specific deacetylation site(s) on IRS1 that are sensitive to Sirt1 and to demonstrate their functional significance. In addition, it should be noted that other insulin signaling molecules such as protein tyrosine phosphatase 1D, phosphoinositide dependent kinase 1 (PDK1), PI3 kinase and SHP1 are also acetylated proteins(22,23) and hence could also possibly be targets of Sirt1-mediated deacetylation. Although previous publications show that Sirt1 deacetylates and activates PGC1-a in peripheral tissues(24,25), we were not able to detect any increase in acetylation level of PGC1-a in SINKO hypothalamus and cultured neurons with Sirt1 inhibition, nor did we observe any changes in levels of PGC1-a target genes in SINKO hypothalamus (data not shown). Our results are in line with what Wu et al has reported in striatum (26). The lack of change in basal PGC-1α acetylation could be due to the absence of a Sirt1 activation signal in our experimental setting. These stimuli, such as caloric restriction or resveratrol treatment, may be required to elicit Sirt1-mediated deacetylation, as previously described (27,28). Alternatively, it is possible that there is differential Sirt1 deacetylation in the CNS.

At first glance, our findings seem to conflict with numerous studies that report beneficial effects of Sirt1 activation on metabolic processes in peripheral tissues. For example, Sirt1 activation in adipose tissue inhibits adipogenesis and induces fatty acid beta-oxidation(29). In islet cells, activation of Sirt1 appears to promote insulin secretion (29), and Sirt1 exerts anti-inflammatory effects in many tissues, including adipose tissue, macrophages and liver(29). Finally, the effects of caloric restriction to increase insulin sensitivity in skeletal muscle are completely dependent on skeletal muscle Sirt1(27) and Sirt1 activation also appears to attenuate hepatic lipogenesis and thereby reduce liver fat content(14). Taken together, these studies support the therapeutic potential for Sirt1-activating compounds in the treatment of metabolic disorders, and clinical trials to test this hypothesis are ongoing.

Our findings suggest that unlike the beneficial effects of Sirt1 activation in peripheral tissues, Sirt1 signaling in the brain has the opposite effect with respect to peripheral metabolic regulation. In this context, a precedent for dual, opposing roles for hormones, enzymes and nutrient-related signals acting in the hypothalamus vs. the periphery is well established. For example, fatty acids can cause insulin resistance via direct effects in peripheral tissues such as liver, but enhance liver insulin action when infused directly into the mediobasal hypothalamus(30). Another example is AMPK, activation of which in peripheral tissues increases fatty acid oxidation and reduces fat deposition, whereas its activation in brain promotes positive energy balance and weight gain(31,32). Similarly activation of malonyl CoA decarboxylase in liver leads to fatty acid oxidation and reduced lipid content(33), while activation of this enzyme in the mediobasal hypothalamus induces hyperphagia and obesity(34).

This phenomenon of opposing actions in the periphery and brain is perhaps best explained by the key role of the hypothalamus in sensing and responding to changes of nutrient availability or energy balance. In the case of AMPK, for example, its activation constitutes a major component of the response to cellular energy depletion and in peripheral tissues, this response mobilizes stored
fuel to meet ongoing energy needs. Activation of AMPK in the mediobasal hypothalamus also constitutes a signal of ongoing depletion of body energy reserves but in this case, behaviors and autonomic responses are mounted to promote positive energy balance, effectively the opposite of what occurs when AMPK is activated in peripheral issues. From this perspective, our current findings raise the interesting possibility that Sirt1 activation in neurons also constitutes a signal of ongoing nutritional depletion and hence induces glucose intolerance and systemic insulin resistance to ensure that glucose is delivered to the brain in adequate amounts by increasing HGP and diverting glucose away from other tissues. This hypothesis warrants additional study.

Our study revealed that adipose tissue de novo lipogenesis is elevated and the epididymal fat pad is larger in SINKO mice. The increased 16:1n7 content is consistent with reduced expression of aP2 and increased expression of SCD-1, as reported previously (16). Although the role of SCD-1 in whole body physiology is complex and tissue specific, adipocyte SCD-1 is highly induced during adipogenesis and serves as an indicator of lipid storage capacity (35,36). Furthermore, adipose SCD-1 suppresses inflammation in vivo (37). Taken together, diminished adipose tissue inflammation and enhanced lipogenesis in SINKO mice may be due to upregulated SCD-1. To this end, it is unclear how CNS Sirt1 regulates adipose lipid metabolism. Several pieces of evidence suggest that altered sympathetic tone might be involved. Recent studies have documented the control of adipose biology by the sympathetic nervous systems (SNS) (4,6,38). Adipose sympathetic denervation significantly increases fat pad size and adipocyte cell number, with increased adipogenesis and lipogenesis (38,39), and pharmacologic sympathetic nervous suppression improves insulin sensitivity (40). Lastly, insulin action in the brain promotes adipose lipogenesis via inhibiting SNS (4). The phenotype of SINKO mice mimics central insulin sensitization and sympathetic blockade, and prompts us to speculate that CNS Sirt1, via SNS, modulates lipogenesis and whole body glucose metabolism. This hypothesis warrants further investigation in the future.

It is also of interest that neuronal Sirt1 deletion caused a striking increase in hepatic insulin sensitivity during HF feeding. This effect suggests that the brain normally constrains liver insulin sensitivity in response to Sirt1-generated signals in key neuronal subsets. This effect of neuronal Sirt1 deletion was not accompanied by changes of hepatic lipid content, including either DAG levels or the individual fatty acid composition of DAGs. It is well known that hepatic DAG levels increase in states of insulin resistance(41), and it has been proposed that DAGs can cause impaired insulin signaling(41). Since HF feeding increased hepatic TAG and DAG accumulation to the same extent in control and SINKO mice, our findings suggest that hepatic insulin sensitivity and hepatic TAG and DAG content can be dissociated. A mild but statistically insignificant reduction \( (p=0.09) \) of ceramide levels was observed in HF-fed SINKO mice. Thus far, it is unclear whether these are direct effects of CNS control on the liver, or they are secondary to the improved adipose inflammation. Regardless, it is probable that less ceramide accumulation could be the cause of hepatic insulin sensitivity in SINKO mice.

Ours is not the first to investigate the role of Sirt1 in the brain and prior findings do not lead to a consensus view of the physiological role for this enzyme in the CNS. Some studies, for example, report that Sirt1 activity protects mice from neurodegeneration(42), although the findings of Li, et al., suggest that Sirt1 may be deleterious to neurons during states of oxidative stress(43). With respect to glucose and energy metabolism, a previous study of the effect of whole brain Sirt1 KO appeared to depend on the age of the mice(11). Studies in which Sirt1 was deleted from hypothalamic POMC, SF1, or MBH neurons more generally point to a positive role of neuronal Sirt1 in whole body metabolism(7,10,44), whereas Wu, et al., reported that overexpression of Sirt1 in the forebrain leads to insulin resistance(26). These studies suggest distinct roles of Sirt1 in different neuronal populations, and in some studies, interpretations appear to be confounded by nonspecific effects of Sirt1 in non-neuronal cell types. One example may be the severe growth retardation phenotype of whole-brain Sirt1 KO mice (generated using nestin-cre, which induces recombination in astrocytes and pituitary cells as well as in neurons)(11). This outcome presumably arises from the absence of pituitary Sirt1 and
consequently, a blunted growth hormone/IGF-1 axis, an outcome that obscures their metabolic phenotype(11). Although our current work does not identify specific neuronal subsets responsible for the insulin sensitive phenotype of SINKO mice, we hypothesize that AgRP neurons may play an important role. This hypothesis is based on our finding that neuronal Sirt1 deficiency increases hypothalamic insulin sensitivity and that insulin signaling in hypothalamic AgRP neurons appears to be necessary for the effect of systemic insulin to fully inhibit HGP(45). In addition, our studies in GT1-7 hypothalamic cells demonstrate a direct, cell-autonomous effect of Sirt1 to reduce insulin signaling, and these same cells are known to express AgRP(46). Since FoxO1 phosphorylation by Akt prevents FoxO1 nuclear translocation and activation(47), our results suggest that Sirt1 deficiency might work through FoxO1 inhibition. Consistent with this hypothesis, deletion of FoxO1 from AgRP neurons induces an insulin-sensitive phenotype resembling that of SINKO mice(48). Efforts to identify the neuronal subpopulations responsible for Sirt1-mediated metabolic effects are an important priority for future studies.

In summary, we report that SINKO mice display increased central and peripheral insulin sensitivity and are protected from diet- and aging-induced insulin resistance. These mice also appear to display enhanced adipose tissue lipogenesis and decreased adipose tissue inflammation. As a known cellular nutrient sensor, neuronal Sirt1 may therefore play a physiological role to detect energy depletion in neurons and trigger adaptive responses that favor increased neuronal glucose availability by increasing glucose production and diverting circulating glucose away from peripheral tissues. Consequently, neuronal deletion of this presumed nutrient sensor protein leads to a favorable metabolic outcome with respect to peripheral insulin action. From a therapeutic perspective, our work raises the novel concept that CNS Sirt1 inhibition, rather than activation, is protective against metabolic diseases and hence those Sirt1 activators that are not brain-penetrating may have greatest therapeutic potential.

Methods:

Animals We backcrossed mice carrying Sirt1 floxed alleles (f/f mice)(49) onto C57BL/6 background for 5 generations. We backcrossed transgenic mice harboring Cre recombinase under the control of the neuron-specific rat synapsin I promoter (Syn–Cre mice)(50) onto the C57BL/6 background for >6 generations. We bred f/f mice with Syn I–Cre mice to generate Sirt1 f/+ mice with a positive Cre gene, which were further bred with Sirt1 f/f mice to obtain Sirt1 SINKO mice. Since occasional male mice carrying synapsin I-Cre can generate germline deletion of the target gene in progeny due to expression of Cre recombinase in sperms, we screened all Sirt1 SINKO mice for germline deletion (e.g., heterozygous whole-body Sirt1 KO mice), and such mice were eliminated from our studies. We housed mice in a 12 h light/12 h dark cycle. At 12–wk of age, mice were either fed normal chow diet (LabDiet, Cucamonga, CA) or 60% HFD (Research Diets, New Brunswick, NJ).

Mouse procedures conformed to the Guide for Care and Use of Laboratory Animals of the US National Institutes of Health, and were approved by the Animal Subjects Committee of the University of California, San Diego.

Metabolic Studies We performed hyperinsulenic euglycemic clamp studies as previously described(12). Since average BW of SINKO mice is lower than control mice, relatively heavier SINKO mice and lighter control mice that have overlapped BW were selected for clamp studies. We only used mice that lost < 6% of their pre–cannulation weight after 4–5 days of recovery. The clamp experiments began with a constant infusion (5 µCi h−1) of D-[3–3H] glucose (Du Pont–NEN, Boston, MA) in 6–h fasted mice. After 90 min of tracer equilibration and basal sampling, we infused glucose (50% dextrose; Abbott) and tracer (5 µCi h−1) plus insulin (6 mU kg−1 min−1) into the jugular vein. We drew small blood samples from the tail vein at 10–min intervals and confirmed the achievement of steady–state conditions (120 mg dl−1 ± 5 mg dl−1) at the end of the clamp by maintaining glucose infusion and plasma glucose concentration for a minimum of 20 min. We took blood samples at t = −10, 0 (basal), 110, and 120 (end of experiment) min to determine glucose–specific activity and insulin concentration. We quantified tracer–determined rates by using the Steele equation for steady–state conditions(51). At steady state, the rate of glucose disappearance, or total GDR, is equal to the sum of the rate of
endogenous glucose productions (HGP) plus the exogenous (cold) GIR. The IS–GDR is equal to the total GDR minus the basal glucose turnover rate.

We performed other metabolic measurements as previously described(12). Immunohistochemistry studies were conducted by the UCSD histology core at Moores Cancer Center.

For assay of biochemical responses to insulin stimulation in the CNS, we injected a bolus of insulin (0.2 U kg\(^{-1}\)) or vehicle via inferior vena cava of anesthetized mice after a 6–h fast. After 10 min, we quickly isolated and snap froze hypothalamus. For assay of biochemical responses to insulin stimulation in peripheral tissues of HF-fed mice, we anesthetized 6–h fasted mice. We ligated vessels supplying one side of leg muscles, one lobe of the liver and one epididymal fat pad and took basal samples of liver, muscle, and fat. Five minutes after a bolus injection of insulin (0.6 U kg\(^{-1}\) via inferior vena cava), we harvested the remaining liver, muscle, and fat and snap froze to measure signal transduction markers.

We performed chronic intracerebroventricular (i.c.v.) cannulation in chow-fed male mice as previously described(52). A stainless steel guide cannula (26 gauge, 7 mm long; Plastics One, Roanoke, VA, USA) was implanted into the right lateral ventricle (anterior posterior: 0 mm (At Bregma); Lateral: 0.8 mm; ventral: 1.8 mm ventral to the surface of the skull) and anchored to the skull with a 1.6 mm stainless steel screw (Plastics One) and dental cement. Mice are allowed to recover for 4 days after cannulation and between experiments. On the day of the experiment, insulin (final concentration 10 mU/µl) was diluted in freshly prepared artificial cerebrospinal fluid (aCSF; 119 mM NaCl, 26.2 mM NaHCO\(_3\), 2.5 mM KCl, 1 mM NaH2PO\(_4\), 1.3 mM MgCl\(_2\), and 10 mM glucose). After 5.5 hr fasting, injections of either aCSF or insulin (10 mU in a volume of 1 µl) were administered via the implanted cannula using an injector (33-gauge, protruding 0.2 mm beyond the tip of the cannula, total length 7.2 mm) connected to plastic tubing and a microsyringe (5 µl) over a period of 1 min. Blood samples were taken before and after i.c.v injection. Thirty minutes after the i.c.v injection, mice were subjected to either OGTTs or ITTs.

Body Composition, Locomotor Activity, and Indirect Calorimetry We measured mouse lean and fat mass using quantitative magnetic resonance spectroscopy (Echo Medical Systems, Houston, TX). We individually placed mice into metabolic cages and adapted all animals to the novel environment for 48 h before study. We normalized VO\(_2\) and VCO\(_2\) using a multiple regression-based approach to account for variation of both lean and fat mass. We calculated energy expenditure (EE) based on EE = 3.815 × VO\(_2\) + 1.232 × VCO\(_2\).

Pl3K Kinase Activity Chow-fed mice were intravenously injected with either vehicle or 0.4 U/kg of insulin. Five minutes later, hypothalamus were quickly dissected and snap frozen in liquid nitrogen. Tissues were then homogenized by in ice-cold buffer containing 25 mM HEPES pH7.5, 137 mM NaCl, 1 mM EDTA, 0.2 mM Sodium vanadate, 10% glycerol, 1% NP-40, and phosphatase and protease inhibitor cocktails. Tissue lysates were subjected to immunoprecipitation for IRS-1 and IRS-1–bound PI 3- kinase activity was assayed in the immunobeads after IP as previously described(27). Immunoprecipitation using normal rabbit IgG, instead of anti-IRS-1 antibody, was used as a negative control. The kinase reaction was initiated with the addition of 2 µg phosphatidylinositol and 20 µCi [γ-32P]-ATP and was allowed to proceed for 30 minutes. After termination of the reaction by the addition of 20 µl 8 N HCL, the lipid products were separated from the remaining [γ-32P]-ATP with 2 successive chloroform extractions and then run for 2 hours on a thin-layer chromatography plate. Separated PI(3)P was visualized on a film.

Cell Culture, Adenovirus Transduction, and siRNA-Mediated Knockdown Immortalized murine hypothalamic cell line GT1-7 cells were described previously(53). Adenoviruses expressing human WT Sirt1 and DN Sirt1 (H364Y) were obtained from Dr. Kaikobad Irani at the University of Pittsburgh Medical Center(20). We cultured GT1-7 cells in DMEM supplemented with 10% fetal bovine serum (FBS). For overexpression or knockdown experiments, we infected the cells with various adenoviruses or treated with siRNA nanoparticles for 5 hrs in cultured media with 2% FBS. The medium was then replaced by fresh DMEM supplemented with 5% FBS for another 24 hrs. Cells were serum starved for overnight in
DMEM with 0.5% FBS before acute insulin stimulation.

**Western Blotting and Immunoprecipitation** We performed Western blotting and immunoprecipitation as previously described (12, 54). We purchased antibodies to β-tubulin, SOCS3, IRS-1, and IRS-2 from Millipore (Billerica, MA). We purchased antibody to p53, PGC1α, and acetylated FoxO1 from Santa Cruz Biotechnology (Santa Cruz, CA) and antibody to Mac-2 from Abcam (Cambridge, MA). All other primary antibodies were obtained from Cell Signaling Technology, Inc. (Beverly, MA). We analyzed the protein bands using Image J densitometry analysis and normalized phosphorylated protein to total protein bands. For protein-protein interaction study, GT1-7 cells were washed with PBS once and then were in vivo crosslinked by 0.1% glutaraldehyde in PBS for 5 min at 37 °C. Crosslink reaction was stopped by adding 1 M Tris-Cl, pH 7.8 to final concentration of 100 mM. Cellular lysates were subjected to immunoprecipitation with a control IgG or an antibody for Sirt1 in a buffer containing 20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 0.1% Nonidet P (NP)-40, 1 mM EDTA, 1 mM PMSF, and protease inhibitor cocktail (Roche, Indianapolis, IN). After incubation at 4°C for overnight, protein A/G agarose beads was added to the immunoprecipitates and incubated for 1 h. The agarose beads were then washed extensively three times before being subjected to Western blot analysis.

**Gene Expression Analyses** We carried out quantitative PCR as previously described (12). Primer sequences used in the PCR reactions were chosen based on the sequences available in GenBank. We measured mRNA of Sirt1 using the following primer set: 5’-GATGCTGTGAAAGTTACTGCAGGAGT-3’ and 5’-GAAGGTCTGGGAGGTCTGGAAG-3’, in which the reverse primer detects exon 4 that is absent in the mutant mice. We normalized the mRNA content of all genes reported to housekeeping genes (cyclophilin A and RNA polymerase II). For detection of mutant Sirt1 mRNA, we used the following primer set that binds to sequence flanking the absent exon: 5’-GATGCTGTGAAAGTTACTGCAGGAGT-3’ and 5’-GAATTTGTGACACAGAGACGGCTGG-3’. Primer sequences for other genes are all exon spanning and are available upon request.

**Quantitative Lipidomics** Quantitative lipidomic studies were performed by Lipomics Technologies Inc. as previously described (17). Ceramide quantity in tissues was measured using the diglyceride kinase assay (55).

**Statistical Analysis** For experiments involving two factors, we analyzed data by two-way ANOVA followed by Bonferroni post tests using Prizm (GraphPad Software, Inc., San Diego, CA). We performed individual pair-wise comparisons using student t test in Excel (Microsoft, Redmond, WA).
Figure Legends

Fig. 1. Neuron-specific deletion of Sirt1 in mice (SINKO).
(A) Quantification of wild type (WT) Sirt1 mRNA in various brain regions of Sirt1^{fl/fl} mice and neuronal knockout (SINKO) mice. Statistical significance between conditions connected by bars is indicated. (B) Quantification of Sirt1 mRNA in various tissues in mice. Statistical significance between conditions connected by bars is indicated. (C) Immunoprecipitation and detection of protein acetylation in hypothalami of control and SINKO mice. (D) RT-PCR showing WT and mutant (KO) Sirt1 mRNA in various tissues in Sirt1^{fl/fl} and SINKO mice. Whole body heterozygous Sirt1 KO mice (f/-) were used as positive controls for exon4 deletion. Statistical significance between caloric restricted control and SINKO mice is indicated. All data are shown as mean ± SEM (n = 6-10/group in all experiments). Gene expression is shown as fold induction normalized with housekeeping gene. Statistical significance is indicated by asterisks (p < 0.05), daggers (p < 0.01), double daggers (p < 0.001).

Fig. 2. Neuronal Sirt1 deficiency causes insulin sensitivity.
(A) Body weight of Sirt1^{fl/fl}, SINKO, and Synapsin I-Cre (Syn-Cre) mice on standard chow or HF diet (HFD). (B) Body composition of control (white bars) and SINKO mice (black bars) before and after 7-wk of HF feeding. (C) Intraperitoneal glucose tolerance tests (IPGTT; 1 g/kg) on 6-month old control and SINKO mice on standard chow. (D) Plasma insulin concentration of 6-month old control and SINKO mice during oral glucose tolerance tests (OGTT; 1 g/kg) on standard chow. Statistical significance between control and SINKO mice is indicated. (E) Fasting or fed serum insulin concentration in mice with either ad libitum feeding (AL) or caloric restriction (CR). Statistical significance between conditions connected by bars is indicated. (F) OGTTs (2 g/kg) on 16-month old control and SINKO mice with either AL or CR. Statistical significance between caloric restricted control and SINKO mice is indicated. (G) Plasma insulin concentration during OGTTs (2 g/kg) in 16-month old control and SINKO mice with CR. Statistical significance between caloric restricted control and SINKO mice is indicated. All data are shown as mean ± SEM (n = 6-10/group in all experiments). Gene expression is shown as fold induction normalized with housekeeping gene. Statistical significance is indicated by asterisks (p < 0.05), daggers (p < 0.01), double daggers (p < 0.001).

Fig. 3. Neuronal Sirt1 deficiency protects mice from HF diet (HFD) feeding-induced insulin resistance.
(A) IPGTTs (1 g/kg) on control and SINKO mice at wk 15 on HFD. (B) Plasma insulin concentration of HFD-fed control and SINKO mice during OGTTs (1 g/kg). (C) Insulin tolerance tests (ITTs; 0.45 U/kg) in control and SINKO mice at wk 16 on HFD. (D) - (G) Hyperinsulinemic euglycemic clamp study on control and SINKO mice fed a standard chow or HFD. Glucose infusion rate (GIR) (D), insulin-stimulated glucose disposal rate (IS-GDR) (E), basal hepatic glucose production rate (basal HGP), insulin-stimulated HGP (F), and percent suppression of HGP (G) are shown. (Inset in D) Matched body weight of control and SINKO mice in clamp studies. (H) – (J) Western blot showing acute insulin-stimulated phosphorylation of AKT in muscle (H), adipose tissue (I), and liver (J). All data are shown as mean ± SEM (n = 6-10/group in all experiments). Asterisk indicates statistical significance between control and SINKO mice, or conditions connected by bars (p < 0.05).

Fig. 4. Increased fat pad size but reduced inflammation in SINKO mice.
(A) Liver and epididymal white adipose tissue (epiWAT) weight of control and SINKO mice on HFD. (B) Functional magnetic resonance imaging of control and SINKO mice on standard chow or HFD. Green arrows indicate liver and red arrows indicate epiWAT. (C) Immunohistochemical image of crown-like structure in epiWAT from HFD-fed control and SINKO mice. Adipose sections were stained for macrophage marker Mac-2 (red). (D) Adipose tissue triglyceride (TAG), diacylglyceride (DAG), and fatty acid content in control and SINKO mice. (E) Adipose tissue gene expression in control and SINKO mice. (F) Serum inflammatory cytokine levels in control and SINKO mice. (G) Liver TAG, DAG, and ceramide content in control and SINKO mice. (H) Liver Socs3 expression in control and SINKO mice. All data are shown as mean ± SEM (n = 6-
10/group in all experiments). Asterisk indicates statistical significance between control and SINKO mice, or conditions connected by bars (p < 0.05).

Fig. 5. Neuronal Sirt1 deficiency sensitizes insulin response in vivo. (A) Western blots showing acute protein phosphorylation in hypothalami of control and SINKO mice. (B) Basal and insulin-stimulated IRS-1-bound PI3K activity in hypothalamus. Neg ctr indicates the negative control. (C) OGTTs (2 g/kg) on 7-month old control and SINKO mice with either intracerebroventricular (i.c.v) injection of vehicle or insulin. Blood glucose levels and plasma insulin concentration are shown as mean ± SEM (n = 4/group), respectively, during OGTTs. Statistical significance between vehicle and insulin injected SINKO mice is indicated (asterisk, p<0.05). (D) Insulin tolerance tests (ITTs; 0.35 U/kg) in 7-month old control and SINKO mice with either i.c.v vehicle or insulin injection. Data are presented as mean ± SEM (n = 4/group). Statistical significance between i.c.v insulin injected control and SINKO mice is indicated (asterisk, p<0.05).

Fig. 6. IRS-1 interacts with Sirt1 and is deacetylated by Sirt1 in hypothalamic cells. (A) Western blots showing insulin signaling in GT1-7 cells infected with adenovirus expressing either eGFP, wild type Sirt1, or dominant negative Sirt1 (H364Y). (B) Insulin-stimulated insulin signaling and acetylated proteins in GT1-7 hypothalamic cells treated with control siRNA or siRNA against Sirt1. (C) Co-immunoprecipitation of endogenous Sirt1 and IRS-1 in GT1-7 cells. (D) Immunoprecipitation and detection of acetylation of IRS-1 in GT1-7 hypothalamic cells infected with adenovirus expressing dominant negative Sirt1.

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Fig. 4

A

Percent Organ Weight

Liver (%) vs epIWAT (%)

B

ff vs SINKO

Chow vs HFD

C

ff vs SINKO

D

mmol/gm

TAG vs DAG vs Myristate (14:0)

Palmitate (16:0) vs Palmitoleate (16:1n7)

E

WAT

Relative mRNA

Tnfa, Il1b, Il6, Ap2, Scd1

F

pg/ml

TNFa, IL10, MCP1, IL6

G

mmol/gm

TAG vs DAG vs Ceramide

H

Relative mRNA

Liver Socs3

ff vs SINKO
Fig. 5

A

|                | Fasted   | Fasted+Insulin | Fed      |
|----------------|----------|---------------|----------|
|                | f/f      | SINKO         | f/f      | SINKO    |
| Sirt1          |          |               |          |          |
| p-IRS (p-Tyr)  |          |               |          |          |
| p-pan PKC (T514) |        |               |          |          |
| p-AKT1/2 (S473) |        |               |          |          |
| p-FoxO1 (S256) |          |               |          |          |
| IRS-1          |          |               |          |          |
| IRS-2          |          |               |          |          |
| AKT1/2         |          |               |          |          |

B

|                | f/f      | SINKO         | Neg Ctr  |
|----------------|----------|---------------|----------|
| Insulin        | -        | +             | -        | +        |

PI3K Activity

Arbitrary Unit

- f/f + Veh
- f/f + Ins
- SINKO + Veh
- SINKO + Ins

*
Fig. 6

A

| p-AKT1/2 (S473) | + + + + + + |
|-----------------|------------|
| AKT1/2          | + + + + + + |
| Myc-Tag         | + + + + + + |
| Insulin         | - + - + - + |
| Ad-eGFP         | + - - - - - |
| Ad-Sirt1 WT     | - - + - + - |
| Ad-Sirt1 DN     | - - - + + + |

B

|                  | Ctr siRNA | Sirt1 siRNA |
|------------------|-----------|-------------|
| Insulin p-IRS (p-Tyr) | 0 5 10    | 0 5 10 (min) |
| p-AKT1/2 (S473)  |           |             |
| AKT1/2           |           |             |
| p-FoxO1 (S256)   |           |             |
| Sirt1            |           |             |

C

IP: Sirt1
IB: IRS-1 or Sirt1

D

IP: IRS-1
IB: Ac-Lysine

Input IP

| M.W. | Ac-IRS-1 | IgG HC | IgG LC |
|------|----------|--------|--------|
| 250  |          |        |        |
| 150  |          |        |        |
| 100  |          |        |        |
| 75   |          |        |        |
| 50   |          |        |        |
| 37   |          |        |        |
| 25   |          |        |        |

Input IP

| M.W. | Nonspecific | IRS-1 | IgG HC | IgG LC |
|------|-------------|-------|--------|--------|
| 250  |             |       |        |        |
| 150  |             |       |        |        |
| 100  |             |       |        |        |
| 75   |             |       |        |        |
| 50   |             |       |        |        |
| 37   |             |       |        |        |
| 25   |             |       |        |        |
Neuronal Sirt1 Deficiency Increases Insulin Sensitivity in Both Brain and Peripheral Tissues

Min Lu, David A. Sarruf, Pingping Li, Olivia Osborn, Manuel Sanchez-Alavez, Saswata Talukdar, Ai Chen, Gautam Bandypadhyay, Jianfeng Xu, Hidetaka Morinaga, Kevin Dines, Steven Watkins, Karl Kaiyala, Michael W. Schwartz and Jerrold M. Olefsky

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