# Improving Insulin Sensitivity With HDAC Inhibitor

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Histone deacetylase (HDAC) has emerged as a new molecular target in the control of obesity and type 2 diabetes. HDAC is an enzyme with well-known functions in the regulation of chromatin structure and gene transcription in the nucleus, where HDAC interacts with corepressor proteins such as NcoR and SMRT to form active corepressor complexes. In the corepressor complex, HDAC catalyzes removal of acetyl groups from histone proteins to inhibit gene expression. Recent studies have consistently suggested that HDAC also exhibits activity in the cytosol and mitochondria to regulate acetylation of metabolic enzymes (1). More than 20% of mitochondria proteins are regulated by acetylation (2,3). Regulation of HDAC activity is a new approach to modify glucose and fatty acid metabolism in the treatment of type 2 diabetes.

In HDAC, HDAC3 and sirtuin (SIRT)1 are well-known players in regulation of fatty acid and glucose metabolism. In mammals, HDACs are divided into three classes: class I HDACs (1–3,8,10), class II HDACs (4–7,9,11), and class III HDACs (SIRTs 1–7 and NAD-dependent histone deacetylases). Class I HDACs have strong catalytic activities, and they are targets of most HDAC inhibitors, such as trichostatin A (TSA), sodium butyrate, and suberoylanilide hydroxamic acid. HDAC3 regulates metabolism in genetic and pharmacological studies. NcoR is required by HDAC3 in the regulation of transcription factors including peroxisome proliferator–activated receptor (PPAR)γ. NcoR knockout in fat tissue led to enhanced PPARγ function in adipose tissue, increased insulin sensitivity, and accelerated weight gain in mice (4), all of which resemble the pharmacological activity of thiazolidinediones. HDAC3 is also involved in circadian-mediated lipid metabolism in liver (5), and hepatic HDAC3 knockout leads to lipid accumulation and glycogen depletion in the mouse liver (6). HDAC3 inactivation in muscle and heart leads to mitochondrial biogenesis deficiency, which reduces fatty acid catabolism in diet-induced obese (DIO) mice (7). Class II HADCs have a weak catalytic activity, and their biological activity is dependent on the class I HDACs. Inhibition of the class I activity will induce suppression of class II. Pharmacological studies suggest that inhibition of class I/II HDACs induces AMP-activated protein kinase (AMPK) activity (8) and has beneficial metabolic effects in humans and rodents (8,9). In contrast, suppression of class III

HDACs generates detrimental metabolic effects (11). Activation of class III HDACs promotes energy metabolism, as is being reported for SIRT1, SIRT3, or SIRT5 (10–12). These activities have been reported for class III HDACs in response to NAD, resveratrol, or gene knockout.

Pharmacological approaches have been used to target class I/II HDACs in regulation of glucose and fatty acid metabolism (Fig. 1). HDAC3 inhibits PPARγ and transcription factor nuclear factor-kB (NF-kB) (13,14). Exchange of HDAC3 is a molecular mechanism of PPARγ and NF-kB cross-talk (13,15). NF-kB activation in inflammation promotes HDAC3 activation, leading to suppression of PPARγ function (15), and HDAC3 inhibition has been shown to restore PPARγ function in obesity (8,15). In one study, two pan-HDAC inhibitors, sodium butyrate and TSA, were supplemented to block HDAC3 activity in DIO mice. The treatment generated a set of unexpected metabolic effects including increased energy expenditure, reduction in adipose tissue expansion, resistance to obesity, and prevention of insulin resistance (8). Mechanistically, AMPK activity and PGC-1α expression were both enhanced in liver and muscle. In a subsequent study, sodium butyrate was found to induce fibroblast growth factor (FGF) 21 expression in liver (16), thereby providing an endocrine pathway for the enhanced energy expenditure in the butyrate-treated mice. Other butyrate derivatives with HDAC inhibitor (HDACi) activity have similar metabolic actions in regulation of insulin sensitivity. Sodium phenylbutyrate alleviated lipid-induced insulin resistance, inhibited endoplasmic reticulum stress, and protected β-cells from failure in obese patients (9). Tributyrin improved insulin sensitivity and inhibited inflammation in DIO mice (17). Inhibition of HDAC4, -5, and -7 (class II) by short hairpin RNA–mediated gene knockdown improved glucose metabolism in DIO mice by suppression of hepatic gluconeogenesis (18). This mechanism is related to downregulation of the transcription factor FOXO1. In these studies, pan-HDACi or class II HDACi was used. Class I HDACi was not tested.

In this issue of Diabetes, the class I–specific (MS275) and class II–specific (MC1568) HDACi were compared in regulation of energy metabolism and insulin sensitivity in DIO mice by Galmozzi et al. (19). Results showed that class I HDACi enhanced whole-body energy expenditure, improved insulin sensitivity, and stimulated oxidative phosphorylation and mitochondrial function in the muscle and fat of mice. The mechanism was attributed to induction of PPARγ coactivator (PGC)-1α. In contrast, class II HDACi did not exhibit these actions, suggesting that class I HDACi are more important in the regulation of energy metabolism and insulin sensitivity. This study provides new insight into the distinction between class I and class II inhibitors in regulation of insulin sensitivity. Despite these interesting new findings, these data should be interpreted with caution. The authors attributed all effects of the class I inhibitor to increased PGC-1α activity (Fig. 1). However, this interpretation is not supported by the phenotypes of PGC-1α overexpression mice. PGC-1α has
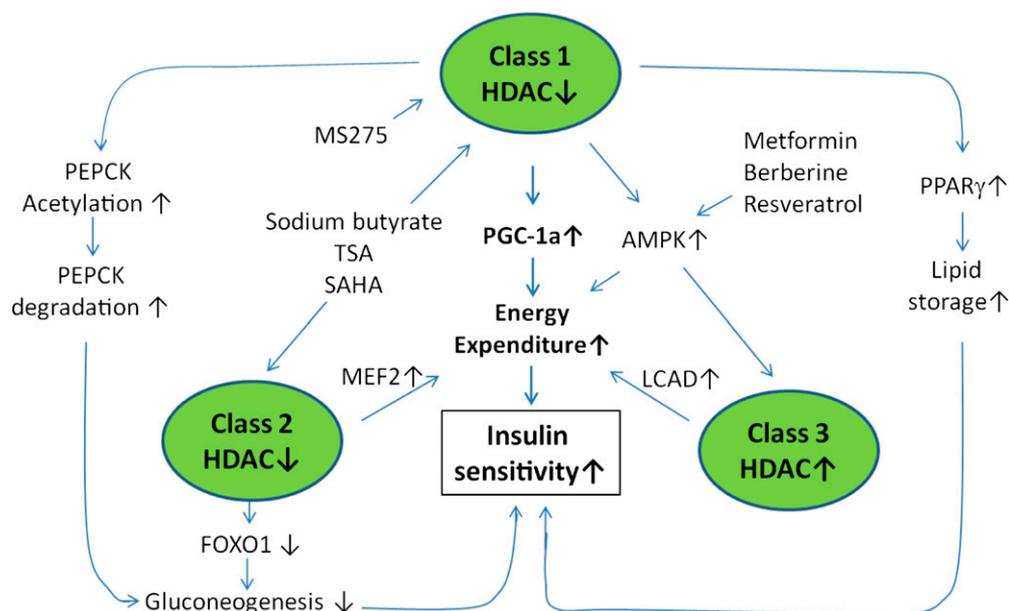
**FIG. 1. HDACi in the regulation of insulin sensitivity. This diagram summarizes mechanisms by which HDACi regulate insulin sensitivity and energy metabolism. LCAD, long-chain acyl-CoA dehydrogenase; MEF2, myocyte enhancer factor 2; MS275, class 1 HDAC inhibitor; PEPCK, phosphoenolpyruvate carboxykinase; SAHA, suberoylanilide hydroxamic acid.**

been studied in two overexpression mouse models for the relationship of mitochondrial function and insulin sensitivity. Muscle-specific PGC-1α overexpression enhanced mitochondrial oxidative phosphorylation, but it did not change whole-body energy expenditure and body weight (20). In this model, the increase in PGC-1α activity made the mice more susceptible to insulin resistance on a high-fat diet. In the other study, global PGC-1α overexpression improved systemic insulin sensitivity modestly (21). At the tissue/organ level, the insulin action was enhanced in muscle but reduced in liver. These studies suggest that activation of PGC-1α by class I HDACi may not be the best mechanism for insulin sensitivity in the current study.

A second consideration is that the role of cytosol protein acetylation was not examined in this study (Fig. 1). Metabolic acetylation is a new mechanism in the control of fuel metabolism in the cytosol. Acetylation, a reaction to add an acetyl group to the lysine residue of a substrate protein, is a posttranslational protein modification that induces changes in protein confirmation and enzyme activity. Acetylation has been found to modify activities of metabolic enzymes in various metabolic pathways in the cytosol (1,22). In the glucose metabolism pathway, acetylation regulates gluconeogenesis (23), glycolysis, glycogenesis, and glucose oxidative metabolism (1). In the fatty acid and amino acid metabolism pathways, acetylation regulates β-oxidation and urea cycle (22). Concentration of glucose, fatty acids, and amino acids determines the acetylation of metabolic enzymes. TSA was shown to regulate the metabolism through protein acetylation in the cytosol in those studies. A final caution is that AMPK activity was not included in the study (Fig. 1). In future studies, isoform-specific HDACi should be tested in the effort to identify new insulin sensitizers in the treatment of type 2 diabetes.

## ACKNOWLEDGMENTS

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