glutamate, may be an important therapeutic approach (Emadi et al., 2014). *MYC* also drives this pathway, although in those studies another enzyme was responsible, the mitochondrial aldehyde dehydrogenase iron-containing enzyme 1 (ADHFE1) and IDH2 (Terunuma et al., 2014). Whether both L and D isoforms were present is not clear, as knockdown of D2HGDH did not affect levels, yet IDH2 knockdown prevented their generation. Tumors with high 2-hydroxyglutarate or DNA methylation patterns had a worse prognosis and high glutaminase expression. It was also shown that glutamine was the main precursor for the 2-hydroxyglutarate. The studies need to be repeated in hypoxia now.

The levels of L-2-hydroxyglutarate or the indirect effects on H3K9me3 methylation could be useful monitors of in vivo hypoxia metabolism and could help classify hypoxia areas of tumors in a different way to using HIF1 or carbonic anhydrase 9 staining or pimonidazole binding (Jubb et al., 2010). Classification of hypoxia in tumors may be helpful for future personalization of radiotherapy and hypoxia-activated prodrugs. Intlekofer et al. (2015) indeed showed that upregulation of H3K9me3 correlated with HIF1 in glioblastoma, but the time course of reversal of L-2-hydroxyglutarate after reoxygenation or demethylation of H3K9me3 is unclear and needs further investigation. For example, if there is discordance of overlap between HIF1α and these pathways in other tumor types, deciphering which of the biological pathways is active in a tumor is important. Individual tumors show great heterogeneity in the extent of hypoxic areas and genes expressed in them, and some with low HIF1α expression could potentially be driven by this metabolic pathway and would produce different types of hypoxia biology, relevant to interaction with drugs and radiation.

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NNMT: A Bad Actor in Fat Makes Good in Liver

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High adipose expression of NNMT, an enzyme that converts nicotinamide to 1-methyl-nicotinamide, correlates with adiposity. Though murine NNMT knockdown in fat and liver prevents weight gain on high-fat diet, Hong et al. (2015) now show that high hepatic expression of NNMT improves lipid parameters via SIRT1 stabilization.

NAD^+ is the central coenzyme for the oxidation of fuel and for interconversion of different classes of metabolites, including the conversion of carbohydrates to lipids (Belenky et al., 2007). NAD^+ is typically reduced to NADH in fuel oxidation steps, whereas NADPH is reoxidized to $NADP^+$ in lipogenic reactions. Cells control major processes such as glycolysis, gluconeogenesis, and lipogenesis, in part, with changes in gene expression programs. Several such programs are modulated by sirtuin 1 (SIRT1), an NAD^+-dependent protein lysine deacetylase (Chang and Guarente, 2014). SIRT1 is not a redox enzyme but rather an NAD^+-consuming enzyme, whose activities as a regulator of gene expression and protein function link lysine deacetylation to the turnover of NAD^+. The products of SIRT1 are a deacetylated

protein plus two metabolites: nicotinamide (Nam) and acetylated ADPribose. Nam, one of three NAD^+ precursor vitamins (Bogan and Brenner, 2008), can be salvaged, i.e., used in resynthesis of NAD^+. However, Nam cannot be salvaged if it is N-methylated by the product of the Nam N-methyltransferase gene (NNMT), thereby forming 1-methyl-nicotinamide (meNam).

Because NAD^+ is required for fuel oxidation and is consumed by sirtuins, the competition between NNMT and NAD^+ salvage suggests that NNMT could be a "bad actor" that might limit fuel oxidation and promote the storage of fat. If NNMT is highly expressed, then Nam might not be salvageable such that NAD^+-dependent processes would be limited. This is exactly what was reported for adipose expression of NNMT (Kraus et al., 2014). In that study, an antisense oligonucleotide that reduced expression of NNMT in liver and white adipose tissue (WAT) allowed mice to resist weight gain on high-fat diet (HFD). Knocking down NNMT in fat cells increased a polyamine catabolic cycle (PCC) gene expression program and oxygen consumption. Interestingly, the meNam product of NNMT had much the same effect as NNMT knockdown in cultured adipocytes, which was attributed to inhibition of NNMT (Kraus et al., 2014).

In a recent paper published in *Nature Medicine*, Hong et al. showed that hepatic NNMT and meNam are "good actors" with respect to protection from some effects of HFD-induced obesity (Hong et al., 2015). Though adipose NNMT expression correlates with adiposity in people and mice (Kraus et al., 2014), Hong et al. found that hepatic NNMT expression correlates with lower serum lipids in mice and morbidly obese people (Hong et al., 2015). Beneficial effects of hepatic NNMT were attributed to the meNam product, which stabilized SIRT1 protein and thereby limited lipogenic gene expression (Hong et al., 2015). The authors found that NNMT knockdown in hepatocytes lowered gluconeogenic gene expression and elevated lipogenic gene expression (Hong et al., 2015). Moreover, NNMT overexpression increased expression of gluconeogenic bypass genes in a manner that was reminiscent of the SIRT1 program (Rodgers et al., 2005). Earlier, pharmacological effects of meNam in adipose tissue were attributed to NNMT inhibition—this was rationalized as a desirable effect because NNMT activity could deplete *S*-adenosyl methionine and NAD^+ cofactors, thereby dampening PCC gene expression (Kraus et al., 2014). Whereas NNMT overexpression depressed NAD^+ levels in WAT (Kraus et al., 2014), such changes were not seen in liver (Kraus et al., 2014, Hong et al., 2015). However, NAD^+ is one of four related NAD coenzymes, and neither study used approaches that quantify changes in the NAD metabolome (Trammell and Brenner, 2013).

If NNMT overexpression and the meNam product do not greatly change hepatocyte NAD^+ but increase SIRT1 signaling, what might meNam do? The breakthrough result was one in which SIRT1 protein stability was increased and polyubiquitylation decreased by meNam (Hong et al., 2015). How meNam blocks SIRT1 polyubiquitylation was not investigated, though it is possible that meNam or a meNam metabolite blocks a ubiquitin ligase. By stabilizing SIRT1, meNam promoted the SIRT1 gene expression program in hepatocytes. In addition, pharmacological meNam supplementation to HFD lowered serum and hepatic lipids, though it did not block weight gain (Hong et al., 2015).

How can NNMT knockdown promote resistance to HFD if NNMT expression and the meNam product also cause some metabolic resistance to the effects of HFD? First, though the antisense reagent dampened expression in WAT and liver, the cell-autonomous effects of NNMT appeared to be stronger in WAT than in liver (Kraus et al., 2014), such that resistance to weight gain was likely mediated by increased oxygen consumption in WAT. Second, because oral meNam may largely be absorbed by the liver in first pass metabolism, it may stabilize hepatic SIRT1 and not be readily available to counteract caspase-dependent degradation of SIRT1 in WAT (Chalkiadaki and Guarente, 2012). Tissue-specific knockdown and overexpression experiments and/or alternative formulations of meNam will be needed to clarify the seemingly disparate results.

It may be helpful to appreciate that NNMT and SIRT1 are neither "bad" nor "good" in all tissues at all times. Animals have deep evolutionary programming to gain weight and store fuel when excess calories are available (Ghanta et al., 2013). The hepatic SIRT1 program, which promotes gluconeogenic gene expression, may be diabetogenic under some conditions and homeostatic under other conditions. Moreover, in WAT, the ability of NNMT to depress NAD^+ salvage may be highly advantageous in order for an animal to maximize seasonal weight gain and protect against famine. We suggest that the tendency of WAT to increase expression of NNMT may aid in fat storage, whereas the activity of NNMT as a SIRT1 stabilizer may allow the liver to cope with the accumulation of body fat produced by episodes of overnutrition.

Experiments involving a genetic or pharmacological intervention added to HFD are unlike the human experience with evidence-based weight loss. Because best available care for overweight conditions consists of reducing energy intake and/or increasing energy expenditure, interventions might be tested in animals coming off, rather than remaining on, HFD. In the context of experimental subjects coming off HFD who are monitored for metabolic health and weight loss, it will be interesting to see whether there are beneficial effects of NNMT knockdown in WAT or increasing meNam in liver and to determine if such interventions can be combined.

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