SIRT1 Takes a Backseat to AMPK in the Regulation of Insulin Sensitivity by Resveratrol

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Resveratrol is a polyphenol that is enriched in the skins of red grapes. Although scientists speculated for decades about the French paradox in which red wine appeared to protect against heart disease, the mechanisms mediating these effects remained a mystery. In 2003 Sinclair and colleagues (1) identified that resveratrol activated the nicotinamide adenine dinucleotide (NAD\(^+\))-dependent histone deacetylase, SIRT1, whose yeast homolog (Sir2) was previously shown to enhance longevity in response to calorie restriction (2). Over the past several years studies in rodents have confirmed the powerful antiaging effects of resveratrol, which have been shown to range from improved exercise performance to correction of age- and obesity-induced dyslipidemia, cardiovascular dysfunction, and insulin resistance (3–5). Whereas SIRT1 has a diverse range of substrates, many of its metabolic actions are believed to be mediated by deacetylation and activation of peroxisome proliferator–activated receptor \(\gamma\) coactivator-\(\alpha\) (PGC1\(\alpha\)), which is a master regulator of mitochondrial biogenesis (4). These studies have suggested that resveratrol and SIRT1 may be the panacea for preventing the development of age-onset diseases.

Resveratrol, like other polyphenols, also increases the activity of the AMP-activated protein kinase (AMPK) (3, 6, 7). AMPK is activated by cellular stress, including fasting and exercise, and is also regulated by circulating hormones and nutrients (8). The activation of AMPK increases fatty acid oxidation, an effect which may involve phosphorylation and inhibition of acetyl-CoA-carboxylase (ACC), reduced levels of malonyl-CoA, and increased mitochondrial fatty acid flux via carnitine palmitoyltransferase-1 (Fig. 1). At the same time, AMPK also phosphorylates and activates PGC1\(\alpha\), resulting in the upregulation of mitochondrial biogenesis (9). The acute activation of AMPK by resveratrol appears to be independent of SIRT1 (10) and is potentially mediated by AMP because resveratrol inhibits the mitochondrial F1 ATPase (11). However, more chronic treatments with resveratrol have suggested that SIRT1 may be upstream of AMPK (12). This idea is supported by the findings that SIRT1 gain of function increases AMPK activity (13), an effect which may be mediated by SIRT1 deacetylation/activation of the upstream AMPK kinase, LKB1 (14).

Although it has recently become appreciated that both SIRT1 and AMPK are evolutionary-conserved metabolic stress sensors whose functions are complementary (15), the finding by Um et al. (16) in this issue of Diabetes suggests that these two pathways are even more inextricably linked than previously appreciated. Using AMPK\(\alpha\)1 and \(\alpha\)2–double-null mouse embryonic fibroblasts, Um et al. demonstrate that resveratrol fails to increase PGC1\(\alpha\) target genes. These studies were surprising because resveratrol was first identified as an activator of SIRT1 from cell-free assays in which AMPK was not present (1). Unfortunately, Um et al. did not assess SIRT1 activity directly by measuring in vitro activity or indirectly by examining deacetylation of substrates other than PGC1\(\alpha\). Therefore, their findings leave us with one of two possibilities: 1) that AMPK is required for SIRT1 activation, which based on previous reports seems unlikely; or 2) that SIRT1 deacetylation of PGC1\(\alpha\) independent of AMPK phosphorylation is insufficient to regulate PGC1\(\alpha\) activity and mitochondrial biogenesis. Future studies will need to examine whether the same is true for other mutual substrates of AMPK and SIRT1, such as forkhead box class O, and potentially other pathways, such as those regulating longevity.

Um et al. (16) expand on these exciting in vitro studies by feeding wild-type or AMPK\(\alpha\)1 and \(\alpha\)2–null mice with a high-fat diet plus or minus resveratrol over a 12-week period. They find that the effects of resveratrol on mitochondrial biogenesis, exercise performance, and whole-body insulin sensitivity are completely dependent on the presence of either AMPK isoform (Fig. 1). Improvements in insulin sensitivity with resveratrol are then linked with reductions in intramuscular diacylglycerol and ceramides, suggesting that resveratrol increased fatty acid oxidation in wild-type but not AMPK\(\alpha\)–null mice (Fig. 1). Consistent with an impaired activation of fatty acid oxidation, Um et al. demonstrate that resveratrol fails to increase the NAD\(-\)NADH ratio or reduce PGC1\(\alpha\) acetylation in skeletal muscle of AMPK\(\alpha\)–null mice, findings that are similar to those of Canto et al. (15), who showed that fatty acid oxidation by AICAR was essential for increasing SIRT1 activity. However, given that previous studies have failed to show inhibition of resting or AICAR-stimulated fatty acid oxidation rates in mice with drastically reduced muscle AMPK activity (17), it seems surprising that the effects of resveratrol on fatty acid oxidation, and therefore the production of NAD equivalents, would be completely eliminated in the AMPK\(\alpha\)–null mice.

An interesting finding from these studies is that both AMPK\(\alpha\)1 and \(\alpha\)2–null mice prevented the induction of mitochondrial biogenesis in muscle and white adipose tissue to an equal degree. This was an unexpected finding given the known expression profiles of the AMPK\(\alpha\) isoforms, where AMPK\(\alpha\)1 is found in all tissues and is predominately in neurons and adipose tissue, whereas the AMPK\(\alpha\)2 isoform is found in skeletal muscle and the heart. Therefore, given that there is <10% reduction in}

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total skeletal muscle AMPK activity in AMPK-α1 mice (18) and AICAR-stimulated glucose uptake (18) and mitochondrial biogenesis (19) are normal, it is astonishing that the effects of resveratrol in muscle are so dependent on the AMPK-α1 isoform. The same scenario also applies to the examination of mitochondrial biogenesis in white adipose tissue, where 95% of total AMPK activity is attributable to the AMPK-α1 isoform (20). These data suggest that resveratrol and AMPK-dependent regulation of metabolism may be cell autonomous. In agreement with this possibility are the findings that resveratrol (5) and SIRT1 activation (21) reduce inflammation. Future investigations using tissue-specific AMPK–double-null mice should help elucidate the primary mechanism(s) by which resveratrol improves insulin sensitivity.

One of the most important questions that remains unanswered is that despite the Internet advertisements touting the health benefits of resveratrol, it is not known whether resveratrol and/or SIRT1 activation is effective in improving insulin sensitivity in humans. If effective, such a therapy would also undoubtedly be highly sought after by endurance athletes looking to enhance performance. However, based on the findings of Um et al., it will also be critical to test whether the new breed of recently developed specific SIRT1 activators (22,23), which do not directly activate AMPK, have a requirement for AMPK in mediating their metabolic and insulin-sensitizing effects. The findings from these studies will be important because they may imply that therapies designed to directly target AMPK, rather than indirectly via SIRT1, may be a more efficacious means to improve insulin sensitivity.

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