engages the sympathetic nervous system. Sympathetic enhancement causes Ca^{2+} loading and Ca^{2+} release-channel phosphorylation, both of which increase EAD/DAD likelihood (Chen et al., 2014). In addition, the associated greatly increased metabolic demand challenges mitochondrially deficient cells, causing them to maximize anaerobic metabolism with the production of large quantities of toxic products typically seen with acute myocardial ischemia (Carmeliet, 1999). These toxic metabolites are free to diffuse from the cell of origin and affect a substantial number of neighboring cells, producing significant amplification and, along with sympathetic enhancement, creating a "perfect storm" for EAD/DAD source generation.

In addition, mitochondrially deficient cardiomyocytes in aged Twinkle mutant mice could also contribute by lowering the electrotonic load in the vicinity of EAD/DAD-producing sources. Under stress conditions, these cells likely lose their capability to generate action potentials and thus no longer constitute a current sink to the EAD/DAD-producing cell(s), decreasing the regional source-sink mismatch. Given that electrotonic influences extend up to 1 mm, it is conceivable that a number of mitochondrially deficient myocytes could be found in the neighborhood of an EAD/DAD-generating source, reducing the electrical sink. In this scenario, rare dysfunctional cells might be understood conceptually as "centers of crystallization" for afterdepolarizations. In the absence of such "crystallization centers," a great majority of emerging afterdepolarization "attempts" would be dampened by electrotonic effects of surrounding cells. Mitochondrially deficient myocytes in the immediate surrounding of "attemptor cells" may significantly increase its chances to propagate and precipitate an arrhythmic event.

Either way, one could conceive of the sporadically distributed mitochondrially deficient cells as "crystallization centers," quiescent at rest but showing enhanced disturbances under stress conditions that alter their electrical properties and affect their aged (and thus not completely normal) neighbors in ways that generate focal arrhythmias.

In conclusion, Baris et al. (2015) have made an important contribution by creating a mouse model of a previously cryptic change in the aging human heart—the appearance of sporadic mitochondrially deficient cells due to accumulated mitochondrial DNA mutations. They demonstrate that reproducing this property of aging predisposes to stress-induced cardiac rhythm disturbances. More work is needed to understand the detailed mechanisms and importance of these electrical abnormalities, but these observations and the availability of this new model will contribute to solving the important enigma of heart disease in the elderly.

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Come to Where Insulin Resistance Is, Come to AMPK Country

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The link between smoking and insulin resistance, despite weight loss, is well established; however, the underlying mechanisms remain elusive. A recent article published in *Nature Medicine* by Wu et al. (2015) reports that nicotine, the main bioactive component of tobacco smoke, activates AMPKα2 in adipocytes, leading to impaired insulin sensitivity.

The subjective experience of almost every smoker is that tobacco affects body weight, and indeed, smokers who try to quit commonly suffer an increase in body mass. This is often reported as a "good reason" to keep smoking, which makes weight gain the main cause of failure in smoking cessation therapies (Filozof et al., 2004). It is well

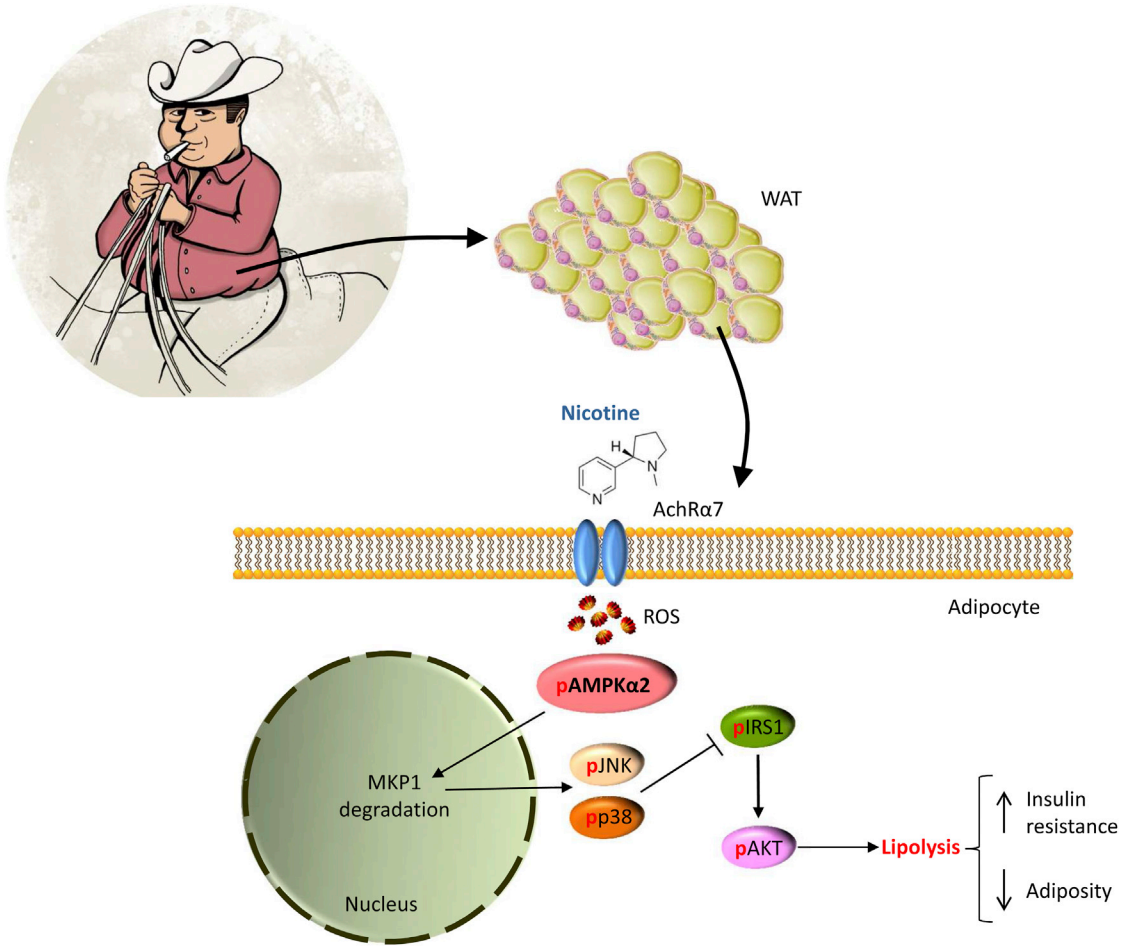

Figure 1. Schematic Representation of Nicotine Actions on White Adipose Tissue to Induce Insulin Resistance through AMPKα2
Nicotine binding to nicotinic acetylcholine receptor subunit α7 (AchRα7) augments ROS levels and increases phosphorylation of AMPKα2, which in turn phosphorylates MAP kinase phosphatase-1 (MKP1), promoting its degradation. This causes impaired activation of p38 mitogen-activated protein kinase (p38) and c-Jun N-terminal kinase (JNK), leading to dysfunction of insulin receptor substrate 1 (IRS1) and inhibition of AKT phosphorylation. This pathway impairs insulin signaling and increases lipolysis.

known that nicotine, the main bioactive component of tobacco, causes smoking-induced changes in body weight. Recent evidence has suggested that nicotine acts in the CNS to induce a catabolic state. Specifically, nicotine activates proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus to decrease feeding (Mineur et al., 2011) and inhibits AMP-activated protein kinase (AMPK) in the ventromedial nucleus of the hypothalamus to increase brown adipose tissue (BAT) thermogenesis through the sympathetic nervous system (Martínez de Morentin et al., 2012; Seoane-Collazo et al., 2014), ultimately leading to weight loss. Ironically, despite this reduction in body weight, nicotine promotes insulin resistance (IR) and hyperinsulinemia in cigarette smokers and in individuals consuming nicotine gum (Eliasson et al., 1996). This is of relevance, as it implies that the use of nicotine replacement therapy during smoking cessation must be temporary and controlled to avoid exacerbated IR in those subjects.

The mechanism by which smoking and nicotine promote IR remains unclear. In a recent paper published in *Nature Medicine*, Yuan, Zou, and colleagues provide new insights into this link (Wu et al., 2015). Using in vitro assays, mouse models, and also human adipose tissue biopsies from non-smokers and smokers, they show that nicotine, acting through nicotinic acetylcholine receptor subunit α7 (AchRα7), induces elevation of ROS levels that activates AMPKα2 in white adipose tissue (WAT), which in turn phosphorylates MAP kinase phosphatase-1 (MKP1), leading to its degradation. Reduction of MKP1 causes anomalous activation of both p38 mitogen-activated protein kinase (p38) and c-Jun N-terminal kinase (JNK), leading to increased phosphorylation of insulin receptor substrate 1 (IRS1) at Ser307. This induces its degradation and inhibition of AKT phosphorylation, ultimately worsening insulin signaling and upregulating lipolysis in WAT (Figure 1). As a consequence of the elevated circulating free fatty acid (FFA) levels in nicotine-treated mice, insulin sensitivity is also impaired in skeletal muscle and liver.

As usual, when exciting data come out, new questions are raised. Although the link between nicotine and AMPKα2 in WAT adds new evidence about its role in the regulation of lipolysis, some questions remain unresolved. The concept that FFAs mediate nicotine-induced resistance is interesting, but mostly based on experiments co-administering nicotine and acipimox (a chemical inhibitor of lipolysis). However, acipimox administration is also linked to other biological effects, such as an increase of growth hormone (GH) secretion, a hormone in itself able to decrease insulin sensitivity (Cordido et al., 1996). It is also likely that nicotine promotes changes in adipokine secretion, such as adiponectin, that could lead to impaired insulin sensitivity. Therefore, systemic measurement of such hormones could offer a more comprehensive understanding of the mechanism. Furthermore, considering that nicotine induces IR in WAT, an increase in macrophage infiltration and inflammation would be expected. However, recent data make that possibility unlikely, since the activation of the AchRα7 by nicotine in obese mouse models in fact enhances insulin sensitivity via suppression of adipose tissue inflammation and macrophage infiltration (Wang et al., 2011). One possible explanation for those discrepancies might be nicotine's dosage and the complexities of nicotine metabolism. Current data have shown that nicotine, when used at higher doses (4 mg kg^{-1} day^{-1} in rats versus 1.5 mg kg^{-1} day^{-1} in mice used in this study), promotes an improvement in insulin sensitivity in association with a catabolic effect characterized by weight loss, increased energy expenditure, lipid utilization, locomotor activity, and elevated BAT thermogenesis (Martínez de Morentin et al., 2012; Seoane-Collazo et al., 2014). Finally, alternative molecular pathways may also be implicated in liver and skeletal muscle. For example, nicotine induces insulin resistance in human skeletal muscle by activating mammalian target of rapamycin (mTOR) (Bergman et al., 2012), a hypothesis that has not been addressed in the current study, whereas no changes in mTOR or its downstream targets were found in WAT.

Although this investigation shows that nicotine specifically activates AMPKα2 in WAT, liver, and muscle, but not in hypothalamus, the actions of nicotine on AMPK are certainly complex. Previous reports show that nicotine inhibits hypothalamic AMPK, lacks effect in muscle, and has a diet-dependent effect on AMPK in liver (Martínez de Morentin et al., 2012; Seoane-Collazo et al., 2014). The reasons for this tissue-specific control are yet unclear and compromise the clinical potential that AMPK-targeting drugs may have in smokers. Given that AMPKα2 is required for nicotine-induced IR, it could be concluded that global or specific inhibition of this kinase in the WAT could represent a new treatment for this disorder in smokers. However, the reality is exactly the opposite: AMPK is a cellular gauge involved in changes in energy status (Kahn et al., 2005), and its activation is one of the mechanisms for the widely used antidiabetic drug metformin (Kahn et al., 2005). Thus, AMPK inhibition for the treatment of nicotine-induced IR seems inconsistent because it could worsen diabetes. Overall, this evidence, besides the clear health benefits of stopping smoking, raises some doubts about the translation of this evidence to clinical practice.

In summary, the study by Wu et al. (2015) provides an important molecular link between nicotine and the promotion of IR through modulation of AMPKα2 in WAT. It also offers new mechanistic evidence about the deleterious effects of nicotine and the healthy benefits of smoking cessation, which will be particularly relevant in smokers suffering from type 2 diabetes. Taken together, these findings further highlight that smoking and nicotine gum are not the ideal strategies for weight control.

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