Putting It Together: Sleep Apnea, the Integrated Stress Response, and Metabolic Dysfunction

Obstructive sleep apnea (OSA) is a very common illness, with prevalence estimates of at least 10% in the general population (1, 2), and considerably higher in certain subgroups, such as those with refractory hypertension or diabetes (3, 4). For years, researchers have recognized the apparent link between OSA and metabolic dysfunction (5). OSA has been independently associated with a variety of metabolic disorders, including elevated fasting glucose, dyslipidemia, hypertension, atherosclerosis, and nonalcoholic fatty liver disease. Although trials of continuous positive airway pressure to treat OSA have led to conflicting results regarding the causal role of OSA in these adverse metabolic outcomes, experimental data from animals and humans suggest that sympathetic overactivation, oxidative stress, and inflammation in OSA may all contribute to the development of metabolic dysfunction (6).

In this issue of the *Journal*, Khalyfa and colleagues (pp. 477–486) suggest that the integrated stress response (ISR) may account for some of the aspects of dysregulated glucose handling observed in OSA (7). The ISR is a eukaryotic signaling pathway that is activated in response to intrinsic and extrinsic stresses such as hypoxia, oncogene activation, viral infection, and endoplasmic reticulum stress, or depletion of necessary ligands such as amino acids and glucose (8). In response to one or more of these factors, various kinases may phosphorylate the α subunit of eukaryotic translation initiation factor 2 (eIF2α), thereby reducing global protein synthesis and aiding in cell survival, mainly via enhanced activating transcription factor 4 (ATF4) expression. There is increasing evidence for the role of the ISR in various lung diseases (9), but there are relatively few data regarding the role it may play in sleep-disordered breathing. OSA may be modeled in animals by a variety of methods. Two commonly used techniques are (*1*) chronic sleep fragmentation, usually using a mechanical apparatus to force repeated arousals from sleep; and (*2*) chronic intermittent hypoxia (IH), which involves the repeated lowering of ambient $F_{I_{O_2}}$ to induce oxyhemoglobin desaturation mimicking that seen in OSA (10). Previous work in the field has demonstrated that each of these animal models of OSA may lead to ISR activation and eIF2α phosphorylation (11, 12), and IH was linked to myocardial infarction via ISR activation in a rodent model (12). However, the role of the ISR in OSA-mediated metabolic dysfunction has not yet been explored.

The authors therefore sought to determine whether disruption of the ISR would protect against IH-induced glucose dysregulation. To that end, they developed a mouse strain with knockout of both GADD34 and CHOP, mediators of the ISR via eIF2α (double-mutant [DM] mice), and additional strains with GADD34 complete knockout and PERK partial knockout (PERK is a kinase that phosphorylates eIF2α to initiate the ISR). These strains were exposed to severe IH (nadir $F_{I_{O_2}}$ 6.4%, 20 cycles per hour, for 12 hours per day for 6 weeks) or room air. Glucose regulation was evaluated by means of the intraperitoneal glucose tolerance test and insulin tolerance test, and visceral white adipose tissue (vWAT) insulin response and immune response were also assessed.

The authors found that IH induced minor but statistically significant weight loss in wild-type animals, but this effect was not seen in any other genotype. Moreover, IH induced dysglycemia during the intraperitoneal glucose tolerance test and insulin tolerance test relative to room-air–exposed mice, but this difference was abrogated in DM mice, suggesting that the ISR may account for these specific IH-induced metabolic changes. Homeostatic model assessment of insulin resistance (HOMA-IR), a product of fasting glucose and insulin levels that is sometimes used to gauge the severity of insulin resistance, was elevated in IH-exposed wild-type mice, but this effect of IH was not observed in DM mice, GADD34$^{-/-}$ mice, or PERK$^{-/+}$ mice. Similarly, vWAT insulin sensitivity, assessed by changes in AKT phosphorylation, was worsened in IH, but again this effect of IH was not observed in DM mice. To help explain these changes, the authors investigated T regulatory lymphocyte and macrophage populations in vWAT, and noted an IH-induced reduction in macrophage number and shift toward M1 phenotype, as well as a reduction in T regulatory cells. No knockout strains showed any of these IH-induced effects. Finally, they evaluated ISR activation at the time the animals were killed by examining eIF2α and ATF4 phosphorylation, and as expected, IH appeared to induce the ISR in wild-type animals but not in any knockout strain.

Taken together, these results suggest that IH may induce metabolic dysfunction via prolonged activation of the ISR. Although the independent effects of OSA on glucose dysregulation have been noted for several years, mechanisms to explain these findings have been relatively less well elucidated. Because OSA is a heterogeneous disease with several potential mechanistic factors (anatomic occlusion, elevated loop gain, reduced arousal threshold, and blunted neuromuscular response to airway occlusion) and with myriad effects (IH and hypercapnia, sleep fragmentation, intrathoracic pressure swings, and sympathetic overactivation), teasing out the specific targets of investigation can be daunting. In this series of experiments, Khalyfa and colleagues advance the field by validating ISR activation in IH as another potential means by which OSA may induce metabolic dysfunction.

As with many novel findings, the new data raise interesting questions and have some important limitations. First and most significantly, in evaluating the metabolic responses to IH, we note that DM mice seem to have abnormal glucose handling at baseline, and that there is a genotype effect independent of IH. Thus, it becomes unclear whether between-group differences are due to the baseline characteristics, potential ceiling effects, or complex interactions of the genetic background and IH exposure. Similarly, although IH did not activate the ISR in DM mice as gauged by eIF2α phosphorylation *per se*, DM mice did seem to have increased baseline eIF2α phosphorylation relative to unexposed wild-type mice. It is unclear whether this may be due in part to the GADD34 knockout specifically, as GADD34 serves to terminate the early phase of the ISR. GADD34$^{-/-}$ mice also

appeared to have higher levels of phosphorylated eIF2α during room-air exposure as compared with wild-type mice in room air. Second, IH has many effects in addition to local tissue hypoxia, such as sympathetic overactivation. Although the characterization of the immune response in different conditions offers hints, the potential role of the autonomic nervous system and other off-target effects in mediating the observed findings is unclear. Third, the ISR response was determined at the time the animals were killed, after 6 weeks of IH exposure. The time course of the ISR response during IH, as well as any compensatory mechanisms that may have contributed to the effects seen, were undetermined.

In summary, the authors demonstrate a potential role for the ISR as a contributing factor in IH-induced metabolic dysfunction, with the caveats listed above. It remains to be seen how this piece may fit with existing literature about the metabolic effects of IH, what the relation may be to human OSA, and whether any pharmacologic targets may arise from this line of inquiry. Given the varying endotypes underlying OSA, one might argue that particular mechanisms underlying OSA pathophysiology may be more or less likely to induce the ISR, depending on the degree of hypoxia and other factors. Only after further mechanistic research into the cardiometabolic consequences of OSA are new therapeutic targets likely to emerge. ■

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