

Shift work is common in healthcare, law enforcement, and other professions requiring 24-hour operations and is associated with circadian disruption and increased consumption of high-fat, high-sugar foods. Circadian disruption (CDR) and a Western-style diet (WD) are known risk factors for systemic inflammation, metabolic syndrome, type II diabetes, cardiovascular disease, and stress-related psychiatric disorders. In this study, we examined whether immunization with *Mycobacterium vaccae* ATCC 15483, a bacterium with anti-inflammatory and stress resilience-promoting properties, could protect against metabolic, behavioral, and neuroinflammatory changes induced by circadian disruption and WD consumption.

Male and female C57BL/6N mice received weekly subcutaneous injections of heat-killed *M. vaccae* ATCC 15483 or vehicle control. Mice were then exposed to either chronic disruption of circadian rhythms or normal light–dark conditions and were fed either a control diet or a WD. Behavioral testing assessed cognitive function and anxiety-like behavior. Fecal samples were collected to evaluate gut microbiome composition, and visceral adipose tissue and hippocampal tissue were collected after euthanasia to assess adiposity and markers of neuroinflammation.

Consumption of a WD altered gut microbiome composition, including an increased Firmicutes:Bacteroidota ratio. Male mice, but not female mice, showed increased body weight gain and visceral adiposity in response to the WD. In males, immunization with *M. vaccae* prevented WD-induced increases in visceral adipose tissue and reduced hippocampal **Nlrp3** mRNA expression, a marker of microglial priming. Immunization also reduced anxiety-like behavior in both sexes under WD and circadian disruption conditions. These findings suggest that *M. vaccae* immunization may mitigate some metabolic, neuroinflammatory, and behavioral effects of Western diet consumption during circadian disruption in a murine model of shift work.