Summary

Since their discovery, antibiotics have revolutionized medicine, providing effective treatment against various bacterial infections. However, while antibiotics target pathogenic bacteria, they also affect beneficial microbiota in the gastrointestinal tract. The gut microbiotas co-evolve with the host and play a critical role in regulating host health, including metabolism, immune function, and neurological health. The overuse of antibiotics, however, can disrupt gut microbiota structure and function, leading to various adverse health effects. This study explores the impacts of a nine-antibiotic cocktail (Neomycin, Streptomycin, Penicillin, Vancomycin, Metronidazole, Bacitracin, Ciprofloxacin, Ceftazidime, and Gentamicin) on the gut microbiota of C57BL/6 male mice through the development from weaning to become young adult. We also investigated the subsequent effects of nine antibiotic cocktail effects on adipose tissue and brain function, particularly focusing on the gut-adipose-brain axis.

Key findings indicate that prolonged antibiotic treatment significantly altered gut microbiota composition. Specifically, Proteobacteria and Actinobacteria populations increased, while Bacteroidetes declined. The shifts were associated with a marked increase in the diversity and decrease in the abundance of gut bacteria, emphasizing the cocktail's effectiveness in disrupting microbiota. Further, we observed increased lipolysis and beiging in white adipose tissue (WAT). Inguinal WAT, for instance, responded faster than perigonadal WAT, reflecting variability in how different fat depots adapt to antibiotic-driven gut microbiota perturbations. Our analysis revealed a positive correlation between the elevated Proteobacteria and Actinobacteria levels and beiging markers, suggesting that microbiota disruption could promote beige adipocyte formation. Additionally, treating adipocyte cell line (3T3L-1) with metabolites

derived from antibiotic-treated mice confirmed the role of microbial metabolites in promoting beiging.

The impact of antibiotic-induced gut microbiota changes extended to brain function and behavior. Behavioral tests, including the Open Field Test, Elevated Plus Maze, Marble Burying Test, and Forced Swim Test, demonstrated that prolonged antibiotic treatment led to increased anxiety and depression-like behaviors in mice. Molecular analyses revealed that antibiotic treatment activated the Hypothalamic-Pituitary-Adrenal (HPA) axis and increased neuroinflammation, which corresponded with reduced neuronal health, and neurotransmitter production. We also observed decreased appetite. The prefrontal cortex, hippocampus, and hypothalamus, all essential for cognitive function, emotional regulation, and appetite control, showed signs of impaired functionality, potentially resulting from the gut-brain axis disruption caused by antibiotics.

Metabolomic studies further confirmed that microbiota-derived metabolites were more abundant than host-derived metabolites in the systemic circulation, with the antibiotic treatment amplifying the trend. We observed that both the host-derived and microbiota-derived metabolic pathways have almost equal effects on developmental-related physiological changes. However, the microbiota-derived metabolites appear to play a central role in regulating host physiology, potentially driving many of the observed changes through the gut-adipose-brain axis following the antibiotic treatment. Our study revealed that prolonged antibiotic treatment can modulate the gut microbiota structure and function, which could influence the host's metabolic and neurological health.