Effects of D-limonene supplementation on bile acid metabolism in obese C57/Bl6 mice.

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D-limonene (DL) is the predominant monoterpene found in orange essential oil, recognized for its antimicrobial, anti-inflammatory, antioxidant, gut-microbiota modulatory, and lipid- and glucose-lowering properties. Bile acids (BA) are metabolized by the gut microbiota and act as signaling molecules, participating in the control of metabolism and inflammation. This study hypothesizes that DL can modulate gut microbiota composition and BA metabolism. To test this hypothesis, an experimental protocol was conducted using male C57/Bl6 mice. The animals were divided into four groups: 1) Low-fat diet-fed mice (NL); 2) High-fat diet-fed mice (HL); 3) HL-fed mice and supplemented with 0.1% DL; and 4) HL-fed mice and supplemented with 0.8% DL. Fecal and liver BA profiles were analyzed by liquid chromatography coupled to high-resolution mass spectrometry. Primary BA comprised approximately 80% of the total BA content in liver and feces. Supplementation with 0.8% DL significantly increased fecal concentrations of total BA ($p<0.005$), while reducing liver concentrations ($p<0.0001$). These results were also observed for primary BA, taurine-conjugated BA, and total conjugates. Western blotting analysis revealed that hepatic FXR (a transcription factor activated by BA) content was 70% higher in the HL 0.8% DL group compared to the NL group, and 40% higher compared to the HL and HL 0.1% DL groups; however, these differences were not statistically significant ($p>0.05$). The hepatic content of TGR5, a G protein-coupled receptor activated by BA showed no response to DL supplementation. This study demonstrates that DL supplementation induces alterations in the BA profile and signaling. These findings provide a basis for future research into the mechanisms by which DL modulates BA metabolism and systemic effects.

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