Measurement of fecal neutral sterol(FNS) excretion and fractional cholesterol absorption(FCA) using the dual fecal isotope method

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Abstract

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Protocol

Step 1.

- A mixture containing 8mg of [26, 26, 26, 27, 27, 27-$^{2}H_{6}$] cholesterol, 8mg of [2, 2, 4, 4, 6-$^{2}H_{5}$] sitostanol was added to 4mL of vegetable oil in a 7mL scintillation vial (this solution requires vigorous stirring for over an hour w/ stir bar before sterols are completely dissolved).
- Using a stainless steel gavage needle attached to a 250µL Hamilton syringe; dose each mouse with 50µL of sterol mixture (when pulling mixture into syringe be sure to remove any bubbles).
- After dosing the mouse, expel any unused mixture back into Eppendorf tube so as to avoid any unnecessary waste.

- Mice are singly housed in wire bottom cages lined w/ paper towels for 72 hours with free access to food and water

- After 72 hours, weigh mice and feces are collected (remove any food particles or paper towel that is attached to feces) and stored in 20mL scintillation vials in -20^{o}C for further analysis.
- Desiccate the feces by placing the vials into a vacuum oven set at 80°C, overnight.

- Weigh the feces to determine the total fecal dry weight.

- Once the feces are dried, they can be stored at room temp and processed at a later date.

Extraction of fecal neutral sterol

Step 2.

- Using a mortar and pestle, crush the feces into a fine powder. Wear a mask and lab coach during this process since the feces contain radioactivity.

- Place 100 µg 5-alpha cholestane into 16x100mm glass screw top tubes. Dry off the solvent under N_2 in the heating block.

- Into the 16x100mm glass tubes, place 100 mg of dried feces and record the exact weight.
- Add 2 ml 95% EtOH and 200 µl 50% KOH to the tube.
- Cap tube and incubate at 60°C for 3 hrs with periodic vortexing.
- Add 2 ml hexane and vortex.
- Add 2 ml water and vortex.
- Centrifuge the tube at 2,700 rpm for 10 min at room temp.
- Transfer the upper hexane phase to a clean 4 mL vial and dry off solvent using N_2.
- Resuspend extract in 50-100µL of hexane and transfer to GC vial for analysis.

$$\frac{\text{(d6-cholesterol/d4-sitostanol dose ratio- d6-cholesterol/d4-sitostanol feces ratio)}}{\text{d6-cholesterol/d4-sitostanol dose ratio}} \times 100 = \text{Percent cholesterol absorption}$$