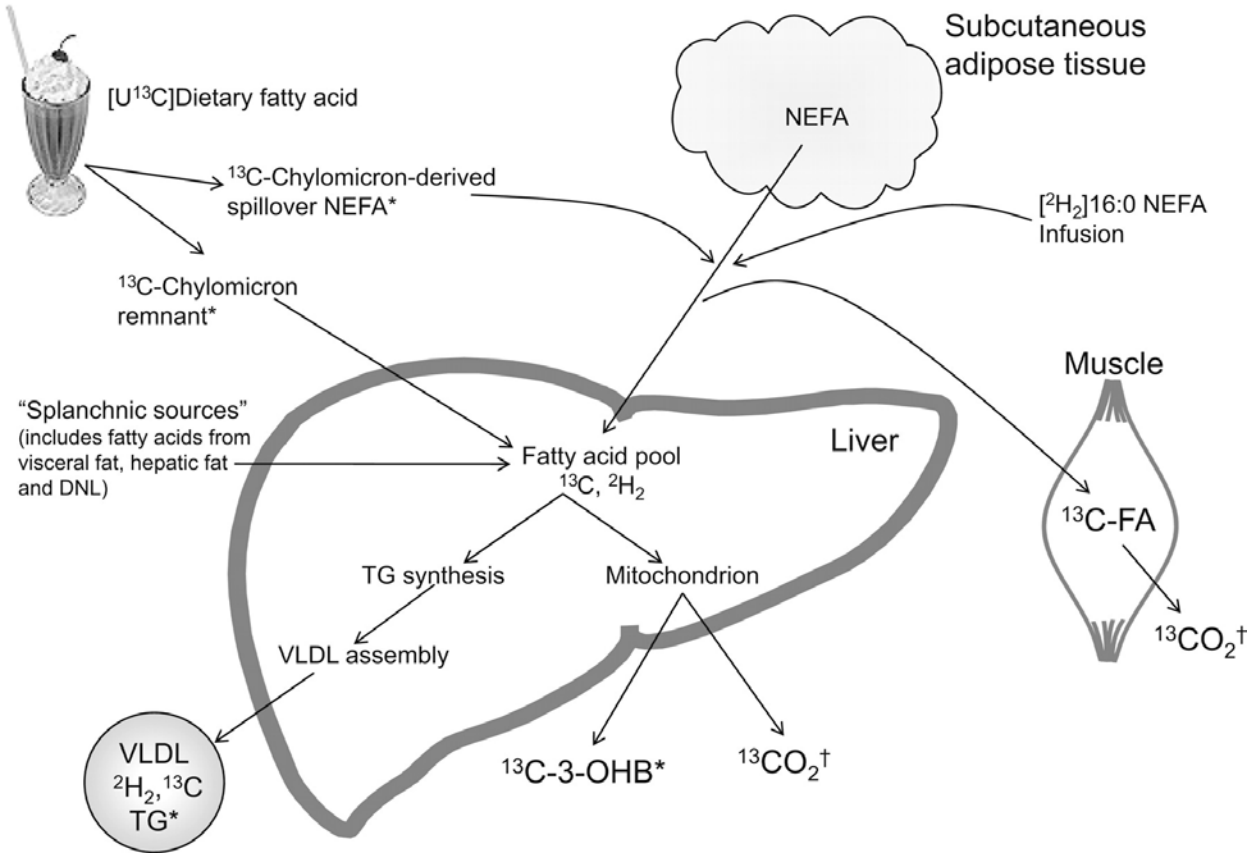
Fatty Acid Metabolism

Measured with Stable Isotope Tracers



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Fatty Acid Metabolism and Stable Isotope Tracers

Lipolysis

Stored triglycerides in the body can be mobilized from fat cells. The process of triglyceride breakdown or lipolysis results in the release of fatty acids and glycerol. Fatty acids can serve as energy substrates while glycerol can act as a gluconeogenic precursor.

Fatty Acid Flux

Isotopic tracers of fatty acids can be used to quantitate the rate of appearance of these substrates into the bloodstream. The continuous infusion-isotope dilution technique of Wolfe et al. (1980) can measure the rate of release of fatty acids into the bloodstream.

Approach

A stable isotope labeled (typically ^{13}C-palmitate) fatty acid is continuously infused intravenously in tracer amounts. The rate of appearance of endogenous unlabeled fatty acids into the bloodstream can be determined by calculating the dilution of the infused isotope. Upon reaching steady-state, the rate of appearance equals the rate of disappearance or uptake. Therefore, the rate of appearance is equal to the flux or turnover rate of the substrate.

Glycerol Flux

The rate of appearance of glycerol is a direct index of lipolysis. Fatty acid flux can underestimate the rate of lipolysis except under fasting conditions because of reesterification. Fatty acids can become reesterified within adipocytes which prevents release of fatty acids into the bloodstream despite active lipolysis. However, glycerol cannot be reincorporated into triglycerides because glycerol kinase is absent within adipocytes.

Approach

A stable isotope tracer of glycerol (typically, D_5-glycerol) is continuously infused. A priming dose of tracer is used to achieve steady-state levels quickly. The stable isotope approach is advantageous compared to radioactive tracer methods because gas chromatography-mass spectrometry (GC/MS) methods measure isotopic glycerol directly. Specific activity of glycerol is difficult to measure because glycerol must be isolated from glucose before counting the radioactivity. GC/MS methods can also be used to accurately measure blood concentrations of glycerol in the same analysis.

Rates of Fatty Acid Futile Cycle

Lipolysis and subsequent reesterification of released free fatty acid represent a futile cycle. This futile cycle allows the adipocyte to rapidly adjust free fatty acid levels in meeting energy demands. Simultaneous isotopic infusions of labeled fatty acid and glycerol tracers will provide an index of the relative rate of fatty acid reesterification. Three fatty acids are released per glycerol molecule released. If triglycerides are hydrolyzed within adipocytes and subsequently fatty acids are reesterified and do not enter the blood stream, then this results in intracellular recycling. The intracellular recycling will be equal to

3 times the flux of glycerol minus the free fatty acid flux. Recycling can also occur when free fatty acids are released into the bloodstream and eventually reesterified. This would be classified as extracellular recycling. Extracellular recycling is calculated as the free fatty acid flux minus the total fat oxidation. Therefore, total recycling equals 3 times the glycerol flux minus total fat oxidation.

Fatty Acid Oxidation

The rate of fatty acid oxidation can be estimated by infusing a C-13 fatty acids and measuring the rate of excretion of expired ^{13}C-CO_2 in the breath. The procedure requires the obtainment of a steady-state level of ^{13}C-fatty acid in the bloodstream and in expired ^{13}C-labeled carbon dioxide. Using priming doses of ^{13}C- sodium bicarbonate before the continuous infusion of tracers will allow isotopic equilibrium by 60 minutes.

Protocols to use for Fatty Acid Flux, Oxidation, Futile Cycles

Best Tracers: 1-^{13}C-Palmitate and D_5-Glycerol
Priming Doses: D_5-Glycerol (1.5 µmol/kg/min)
1-^{13}C-Palmitate (none)
1-^{13}C-Sodium Bicarbonate (0.07 mg/kg)
Infusion Pump Speed: 0.174 cc/min
Infusion Rate: Glycerol (0.10 µmol/kg/min)
Palmitate (0.04 µmol/kg/min)
Sampling Times: 0, 60, 70, 80, 90 min. (Plasma and Breath)
Diet Protocol: Fasted or Fed
References:
Wolfe et al., Biomedical Mass Spect. 7:168-171, 1980
Shaw and Wolfe, Ann. Surg. 205:368-376, 1987
Wolfe and Peters, Am. J. Physiol. 252:E218-E223, 1987
Klein et al., Am. J. Physiol. 257:E65-E73, 1989

Preparation of Tracer

Glycerol is infused as a sterile pyrogen-free solution. Normal saline is used to dilute the glycerol to the appropriate concentration. Before infusion of palmitate, the tracer must be bound to albumin. The palmitate-albumin mixture is prepared by first dissolving a known quantity of palmitate in hexane, using sterile containers. Use enough hexane to completely dissolve the palmitate. An equimolar quantity plus 3% excess of KOH (dissolved in 80% methanol) is added to the hexane solution. The solution is evaporated to dryness with nitrogen using a heated water bath (or sand bath) at 60 °C. Preheated (60 °C) sterile water is added to the dry potassium salt of palmitate. Use enough water to solubilize the dry salt. Transfer the aqueous solution with a heated (60 °C) sterile syringe attached to a Millipore™ (0.22 micron) filter to a bottle of sterile human albumin (Cutter Laboratories, Emmeryville, CA).

Calculations

Ra (μmol/kg/min) = $(E_i/E_p - 1) \times I$

where Ra = rate of appearance of substrate, E_i = Enrichment of infusate (atom % excess, APE), E_p = Enrichment of substrate in plasma (APE), and I = infusion rate (μmol/kg/min).

FFA oxidation (μmol/kg/min) = $(E_b \times VCO_2 \times 16)/(E_p \times k \times \%\ palmitate)$

where E_b = enrichment of breath CO_2, VCO_2 = μmol/kg/min Ventilation rate, E_p = enrichment of palmitate in plasma, k = correction factor for retention of bicarbonate in blood (0.81) and % palmitate = the % palmitate concentration in blood.

Intracellular Recycling = 3 x Ra glycerol - Ra FFA

Extracellular Recycling = Ra FFA - Total Fat Oxidation (indirect calorimetry)

Total Recycling = 3 x Ra glycerol - Total Fat Oxidation