Two-step hyperinsulinemic euglycemic clamp

Subjects were admitted to the Metabolic Clinical Research Unit of the AMC after an overnight fast and studied in the supine position. A catheter was inserted into the distal vein of each arm. One catheter was used for sampling of arterialized blood using a heated hand box (55 C). The other catheter was used for infusion of [6,6-$^2H_2$]glucose, glucose 20%, and insulin. At 09:00 h (t = −2.0 h), after drawing a blood sample for background enrichment of plasma glucose, a continuous infusion of [6,6-$^2H_2$]glucose (99% enriched; Cambridge Isotopes, Andover MA, USA) was started at a rate of 0.11 µmol/kg·min after a priming dose equivalent to 80 minutes. After an equilibration time of 2 hours, blood samples were drawn for glucose enrichments, FFA and glucoregulatory hormones. Thereafter a continuous infusion of insulin (Actrapid 100U/ml; Novo Nordisk Farma, Alphen a/d Rijn, The Netherlands) was started for 2.0 h at a rate of 20 mU/m^2 body surface area·min. Plasma glucose was measured every 10 minutes and glucose 20% enriched with 1% [6,6-$^2H_2$]glucose was infused at a variable rate to maintain plasma glucose at 5.0 mmol/L. At t = 2 h, blood samples were drawn for glucose enrichment, glucoregulatory hormones and FFA. Thereafter the infusion of insulin was increased to a rate of 60 mU/m^2 body surface area·min and the blood drawings were repeated at t= 4 h.

Laboratory analyses

Plasma glucose concentrations were measured with the glucose oxidase method using a Biosen C-line plus glucose analyzer (EKF Diagnostics, Barleben/Magedeburg, Germany). Insulin and cortisol were determined on an IMMULITE 2000 system (Siemens Healthcare Diagnostics B.V., Breda, The Netherlands). Cortisol was determined with a chemiluminescent immunoassay (intra-assay variation 7–8%; total-assay variation 7–8%; detection limit 50 nM). Insulin was determined with a chemiluminescent immunometric assay (intra-assay variation 3–6%; total-assay variation 4%; detection limit 15 pM). FFA were measured by an enzymatic method (Nefac; Wako Chemicals, Richmond, VA, USA) (intra-assay variation 1%; total-assay variation 4–15%; detection limit 0·02 mM). Leptin was determined with a human leptin radioimmunoassay kit (Millipore) (intra-assay variation 3–8%; total-assay variation 3–6%; detection limit 0·5 ng/ml). Glucagon was determined with the Linco 125I radioimmunoassay (Merck Millipore) (intra-assay variation 9–10%; total-assay variation 5–7%; detection limit 15 ng/l). [6,6-$^2H_2$] glucose enrichment was measured with gas chromatography-mass spectrometry as described in detail earlier (1).

Reference List

1. Ackermans MT, Pereira Arias AM, Bisschop PH, Endert E, Sauerwein HP, Romijn JA. The quantification of gluconeogenesis in healthy men by (2)H2O and [2-(13)C]glycerol yields different results: rates of gluconeogenesis in healthy men measured with (2)H2O are higher than those measured with [2-(13)C]glycerol. J Clin Endocrinol Metab 2001 May;86(5):2220-2226.