Assessing postprandial glucose using the triple tracer technique

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Glucose homeostasis is maintained in healthy individuals due to a complex orchestration of a number of key hormones, metabolites and organ systems. Dysregulation of this glucose homeostasis is the main dysfunction associated with Type 2 Diabetes (T2D), a globally emerging health concern estimated to effect almost 400 million adults (Guariguata *et al.*, 2014). Therefore, obtaining an accurate and physiologically relevant description of glucose metabolism is essential in order to further our understanding of the pathophysiology of T2D. However, much of the scientific knowledge regarding glucose metabolism is inferred from non-physiological measurement techniques. This is especially true in regards to postprandial glucose metabolism, where the non-steady state resultant from meal ingestion makes accurate assessment of glucose flux challenging. The 'gold standard' technique to measure glucose flux accurately under physiological postprandial conditions is the triple tracer technique (Dalla Man *et al.*, 2013), but so far it is underutilized in human research. Therefore, our research aims to 1) optimize the administration of the triple tracer technique in humans to then 2) examine the relative contributions of metabolically relevant tissues to whole body glucose metabolism following a single bout of acute exercise and 4 weeks of endurance exercise training.

Four healthy, lean males (aged 18-35) were recruited into a pilot study to initially optimize the triple tracer technique for the subsequent intervention trials. After an overnight fast, participants arrived at our lab and began a primed-continuous infusion of [1-13C]glucose. After 150 minutes, participants ingested a mixed meal (10kcal kg^{-1}, 45% carbohydrate, 15% protein, and 40% fat) consisting of eggs, cheese and Jell-O (1.2g kg^{-1} body weight dextrose) containing [6,62H2]glucose at an enrichment of 4%. With the first bite of the meal, [1-13C]glucose infusion was altered in a pattern so as to approximate the anticipated fall in endogenous glucose production (EGP) and [U-13C6]glucose infusion was started in a pattern so as to mimic the anticipated rate of appearance (Ra) of the [1-13C]glucose in the meal. The rate of tracer infusion was determined based on *a priori* knowledge and from previous studies using glucose tracer infusion (Sathananthan *et al.*, 2015). Blood was sampled at regular intervals and glucose tracer concentration was determined *via* gas chromatography mass spectrometry. Tracer-to-tracee ratios obtained from each participant were used to inform the tracer infusion rate in the subsequent participant. This approach allowed us to optimize the infusion pattern of the intravenous tracers and therefore minimize tracer-to-tracee ratios and derive model-independent measures of glucose rate of disappearance (Rd), Ra and EGP.

In the exercise trial, body composition scans and triple tracer trials were undertaken before, after a single bout and after 4 weeks of endurance training. Exercise was completed at 70% VO_2max for 60 minutes, 5 days per week, with the intensity increased weekly, so as to maintain the same relative intensity despite increases in fitness. Currently, one participant has completed both the acute exercise bout and exercise training protocol. Despite considerably improved glucose tolerance following both acute exercise and exercise training, glucose fluxes (glucose Ra, Rd and EGP) remained unchanged. This is in concurrence with previous research, which showed unchanged glucose fluxes despite significant improvements in glucose tolerance following caloric restriction in participants with T2D (Sathananthan *et al.*, 2015). Once all data has been obtained, measures of glucose flux will be incorporated into variations of the Oral Minimal Model along with insulin, glucagon and C-peptide in order to determine hepatic and peripheral insulin sensitivity, glucose effectiveness, β-cell function and insulin action (Cobelli *et al.*, 2014). From this we will determine the relative contributions of metabolically relevant tissues to changes in whole body glucose metabolism. We hypothesize that improvements in both hepatic and peripheral insulin sensitivity will contribute to the significantly improved whole body insulin sensitivity and glucose tolerance.

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