

Pilot Study: Determining timing of isotopic & metabolic steady state following exercise

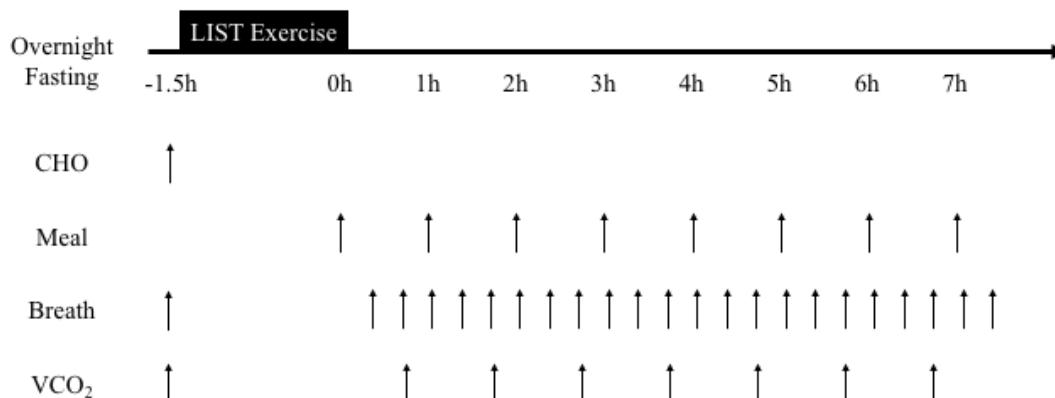
Purpose: The purpose of this pilot study was to determine whether our participants reach isotopic- and metabolic- steady state (i.e. plateau in breath $^{13}\text{CO}_2$ enrichment and VCO_2 production) without a prior adaptation period. Although a previous study demonstrated that no adaptation was needed, that study did not employ an exercise stimulus prior to feeding (Humayun et al, 2007). Therefore, we examined the trend of $^{13}\text{CO}_2$ excretion and VCO_2 production following our modified version of the Loughborough Intermittent Shuttle test (LIST, described in detail in main study document), using hourly meals with identical composition as the main study, except without addition of tracer.

Methods: Three participants took part in the pilot study (**Supplemental table 1**). Participants completed the same protocol as the main study, except samples were collected more frequently (see **Supplemental figure 1**) to identify the time-course of achievement of steady-state, and the meals contained no added tracer. The diet contained 1.2g PRO/kg BW/d, 6.0 g CHO/kg BW/d, and total energy content equal to $(\text{RMR} \times 1.5) + 0.1429 \text{kcal/min/kg} \times \text{BW} \times 75 \text{min}$ (estimated EE during the LIST). Participants arrived at the lab after an overnight fast, and consumption of a carbohydrate beverage breakfast which provided energy for the exercise portion (1g/kg CHO). The first study meal was provided immediately upon completion of the exercise protocol. VCO_2 measures were collected during 20-min continuous intervals while seated in a quiet room, with a baseline measure occurring prior to exercise, and 7 hourly measures beginning 40-min after the first meal. Breath samples were collected in exetainer tubes at baseline and at 20-min intervals beginning 20-min after the first meal.

Supplemental table 1. Participant Information

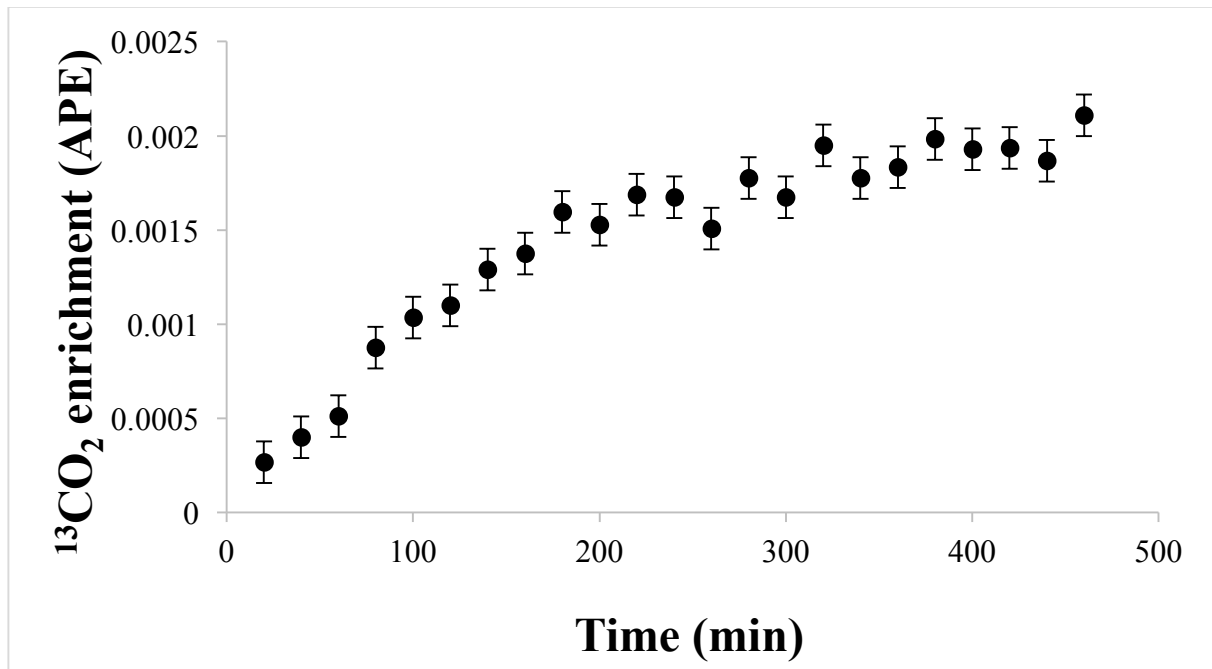
Age, yr	20 ± 1.15
Height, cm	168 ± 4.9
BW, kg	65 ± 4.8
RMR, kcal/d	1645 ± 61.7
Average Daily EE, kcal/d	2910 ± 400

Supplemental figure 1. Study Protocol Outline. Three individuals completed the pilot study with a single test protein intake (1.2g/kg/d).

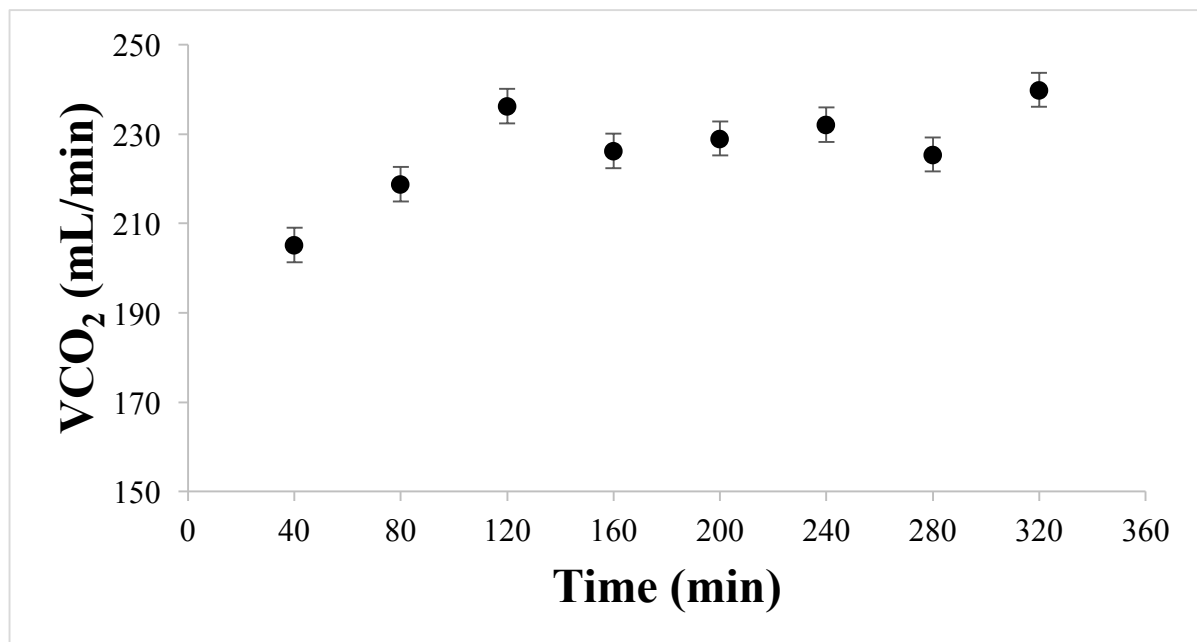


Statistical Analysis: The change in background $^{13}\text{CO}_2$ enrichment from breath samples was determined using regression analysis (Graphpad Prism® 5; GraphPad Software, Inc., La Jolla, CA). Establishment of isotopic steady state was evaluated by repeated linear regression analysis in which data points, beginning at time 0 min, were removed until a regression line with a slope not different from zero was achieved.

Results: Changes in breath $^{13}\text{CO}_2$ enrichment and VCO₂ production following exercise and hourly meals are depicted in **Supplemental Figures 2 & 3**. The slope of the $^{13}\text{CO}_2$ enrichment 300 min after consuming the first hourly meal was not significantly different from zero ($P > 0.05$). The slope of the VCO₂ 100 min after consuming the first hourly meal was not significantly different from zero ($P > 0.05$).



Supplemental figure 2. Time course of $^{13}\text{CO}_2$ breath enrichment following the LIST with hourly feedings. Slopes of the $^{13}\text{CO}_2$ enrichment vs. time regression lines were not significantly different from zero 300 min after the completion of the exercise ($P > 0.05$) indicating a plateau was achieved. Values are means \pm SEM, $n = 3$.



Supplemental figure 3. Time course of VCO_2 following the LIST with hourly feedings. Slopes of the VCO_2 vs. time regression lines were not significantly different from zero 100 min after the completion of the exercise ($P > 0.05$) indicating a plateau was achieved. Values are means \pm SEM, $n = 3$.

References

Humayun, M. A., Elango, R., Ball, R. O., & Pencharz, P. B. (2007). Reevaluation of the protein requirement in young men with the indicator amino acid oxidation technique. *The American journal of clinical nutrition*, 86(4), 995-1002.