**S1 text. The pilot study to determine an isotopic and metabolic steady state during metabolic trial.**

**Purpose**

The objective of this pilot study was to determine whether an isotopic steady state could be reached in breath 13CO2 enrichment without prior adaptation to the study meal. This was examined by determining the pattern and stability of background 13CO2 enrichment in breath and VCO2, while subjects consumed the experimental diet without containing L-[1-13C]phenylalanine.

**Methods**

Four subjects took part in this pilot study (**Supplemental** **Table 1**). The study design was based on the previous study (1) modified with an addition of the exercise stimulus (summarized in **Supplemental Figure 1**). The protocol was same as the protocol in our metabolic trial day except for the sample collection and the test diet. The test diets supplied 1.4 g protein/kg BW/day, 9.0 g Carbohydrate/kg BW/day and 1.6 \* resting energy expenditure (REE) plus the exercise-induced energy expenditure (EEE) without substituting L-[1-13C]phenylalanine. Breath samples were collected every 30 min from 15 min to 465 min after ingesting the first hourly meal to determine 13CO2 enrichment (summarized in Supplemental Fig.1). VCO2 was measured over 20-min continuous intervals every 60 min from 460 min after ingesting the first hourly meal.

**Supplemental Table 1** Characteristics of participants

|  |  |
| --- | --- |
|  | Mean ± SD |
| Age, yr | 29.0 ± 5.0 |
| Height, cm | 174.5 ± 4.4 |
| Body weight, kg | 68.6 ± 9.1 |
| Fat-free mass, kg | 59.6 ± 8.5 |
| VO2peak, ml/kg/min | 59.5 ± 5 |
| REE, Kcal/day | 1745.1 ± 221.2 |

4 participants participated in this study.

REE, resting energy expenditure

**Supplemental Figure 1. The protocols employed for each metabolic trial.** 4 subjects performed metabolic trial once per subject.



**Statistical analysis**

The significance of the change in background enrichment of 13CO2 over the 8 h on the metabolic trial was tested by 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**

The effect of hourly meals on background 13CO2 enrichment in breath and VCO2 after exercise stimuls was shown in **Supplemental Figure 2, 3**. The slope of the 13CO2 enrichment 285 min after consuming the first hourly meal was not significnatly different from zero (P > 0.05). VCO2 was kept constant after 20 km run until the end of the metabolic trial.



**Supplemental Figure 2 Effect of exercise and hourly meal on enrichment of 13CO2 in breath.**

A plateau in 13CO2 enrichment was achieved at 285 min .Slopes of the 13CO2 enrichment vs. time regression lines were not significantly different from zero 285 min after the completion of 20 km run ( P > 0.05). Values are means ± SEM, N = 4.



**Supplemental Figure 3 Effect of exercise and hourly meal on VCO2.**

Slopes of the VCO2 vs. time regression lines were not significantly different from zero. Values are means ± SEM, N = 4.

**References**

1. Bross R, Ball RO, Pencharz PB. Development of a minimally invasive protocol for the determination of phenylalanine and lysine kinetics in humans during the fed state. The Journal of nutrition. 1998;128(11):1913-9.