**Supplemental Materials**

*Exercise Testing*

Following body composition assessment, participants completed a graded exercise test on a cycle ergometer (Monark LC6 novo; Varberg, Sweden) to measure maximal oxygen consumption (VO2MAX). During cycling, expired gases were measured using a breath-by-breath metabolic cart (Cosmed Quark CPET; Rome, Italy), and heart rate was monitored using a chest strap (Polar H10; Kempele, Finland). All participants completed three 180-second stages of 25, 50, and 75W each before resistance increased by 25W per minute to volitional exhaustion. Breath-by-breath data were averaged into five-second bins for analysis. Because we did not formally assess rating of perceived exertion (i.e., via Borg RPE) or measure blood lactate, we used three criteria to determine attainment of VO2MAX vs. VO2PEAK: a plateau in VO2 ≤ 50 mL/min in the last 30 seconds of exercise, a heart rate within 10 beats/min of age-predicted maximum (208 – 0.7\*age in years), and a peak RER ≥ 1.10. Of the 27 participants included in the final analysis, 19 met at least two out of three criteria and were considered to have reached VO2MAX. Of the remaining eight, two reached an RER ≥ 1.05 and a plateau in VO2 but did not reach an RER of ≥ 1.1.

*Metabolic calculations*

Fat oxidation was calculated as [VO2 (L/min) x 1.67) – (VCO2 (L/min) x 1.67)] and carbohydrate oxidation as [ VCO2 (L/min) x 4.55) – (VO2 (L/min) x 3.21)]. Matsuda Index was calculated as 10,000/√ [fasting glucose (mg/dl) × fasting insulin (µIU/mL)] × [mean glucose (mg/dl) × mean insulin (µIU/mL) during the OGTT before normalizing to the 100g dose. Adipose tissue insulin resistance (Adipo-IR) was calculated as fasting insulin (µIU/mL) \* fasting NEFA (mEq/L) and using postprandial OGTT values (30, 60, 90, 120) as mean plasma NEFA (mEq/L) x mean serum insulin (µIU/mL). Fasting insulin and glucose measurements were used to calculate the homeostatic model assessment of insulin resistance (HOMA-IR)) as [fasting insulin (µIU/mL) - (fasting glucose (mg/dl)/18.156)] / 22.5.

*Model fitting*

To account for the possible nonlinear effect of time on postprandial metabolism (i.e., a nonlinear change in metabolism), models with an additional quadratic time term and quadratic time x BF% were considered. Models with and without the quadratic time terms were compared using likelihood ratio tests to determine if the quadratic terms should be retained. Each variable included in the models was standardized to a mean of 0 and standard deviation of 1, including time. Fasting and postprandial variables were standardized separately due to the inclusion of the fasting state as a covariate in the models. Variables in the models were tested for normality with the Shapiro-Wilk test and transformed following confirmation by visual examination. As a result, insulin and NEFA concentrations were transformed via natural log, and VAT volume was transformed via cube root. In models where the quadratic model was retained, we considered either a significant time\*BF% or a time2\*BF% interaction as evidence of a significant interaction. For models where both interaction terms were not significant, we refit the model without interaction terms.

*Effect of body composition on substrate response*

Using mixed-effects models, we tested the effect of BF% on glucose, insulin, and NEFAs over time and time2 to account for a potential nonlinear response. To interpret our results, obtained beta values were multiplied by 8.7%, the standard deviation for body fat percentage in our sample, to demonstrate the expected changes in blood glucose, serum insulin, and serum NEFAs based on the standard deviations for those measures (**Table S2**). The addition of a time2 main effect and a time2 x BF% interaction did not improve the model fit for the effect of BF% on postprandial blood glucose (p = 0.237) or insulin (p = 0.545), suggesting that a linear model has adequate fit relative to a quadratic model. In the linear models without time2, BF% did not modify the relationship between time and glucose (p = 0.294) or insulin (p = 0.178). In the subsequent main effects models, a 7.0% increase in body fat percentage would result in a significant (β = 0.802; 95% CI: -0.021, 1.630; p = 0.055) 19.1 mg/dl increase in postprandial blood glucose at each time point. A 2.0% change in body fat is associated with a non-significant increase in serum insulin (β = 0.232; 95% CI: -0.285, 0.749; p = 0.355). For NEFAs, model fit was improved by the addition of a time2 main effect and a time2 x BF% interaction (p < 0.001) compared to the model with only linear time terms. In this quadratic model, BF% modified the relationship between NEFAs and time (p < 0.001) but not time2 (p = 0.313); the resulting model is plotted in Figure S2, showing an association between BF% and delayed suppression of lipolysis. Thus, body composition affects postprandial blood glucose and NEFAs but not serum insulin throughout the OGTT (**Table 3**).

*Accounting for differences in fat-free mass*

Because skeletal muscle is a primary site of postprandial glucose disposal1 and people with obesity often have higher fat-free mass2, we tested the effect of adding fat-free mass as an additional covariate. This did not improve the model fit (p = 0.204) over the original CHO model. Like the original CHO model, the addition of a time2 main effect and a time2 x BF% interaction term improved the model fit (p = 0.002), and percent body fat modified the relationship between CHO and time2 (p = 0.023) but not time (p = 0.182). Like RER and CHO, higher BF% was associated with greater CHO adjusted for FFM, indicating differences in FFM across our sample do not affect the interaction between time and BF%.

*Robustness of results*

To better understand potential variability in our results, we calculated the 95% confidence interval for the fixed effects in our models using bootstrapping with resampling by subject. We used lmeresampler::bootstrap with type = “case” to conduct bootstrapping with 1000 replicates. Confidence intervals were then calculated using the confint function and the percentile method for comparison to the original Satterthwaite confidence intervals in **Table S5**. Unfortunately, we were unable to calculate Satterthwaite CIs from bootstrapped data and have calculated profile confidence intervals instead. This may artificially inflate the differences between original and bootstrapped confidence intervals.

We also considered the potential for overfitting and over-adjusted models by testing models with adjustment for sex, Matsuda Index, and the fasting measure as our only covariates. In these analyses for the effect of BF% on RER, a quadratic model was still a better fit to the data (p=0.002). In this quadratic model, BF% modified the relationship between time2 and RER (p = 0.0188) but not time (p = 0.187). In these analyses for the effect of BF% on CHOX, we observed similar results; a quadratic model was a better fit to the data (p = 0.002), and BF% modified the relationship between CHOX and time2 (p = 0.023) but not time (p = 0.182). These results suggest our primary models were not overfit based on inclusion of additional covariates.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Supplementary Table 1. Participant Anthropometric Characteristics by Biological Sex | | | | |
| **Variable** | **All (n = 27)** | **Female (n = 15)** | **Male (n = 12)** | **p-value (Sex)** |
| NIH Race/Ethnicity (%) |  |  |  | 0.902 |
| Black | 7 (25.9%) | 4 (26.7%) | 3 (25.0%) | --- |
| Hispanic or Latino | 1 (3.7%) | 0 (0.0%) | 1 (8.3%) | --- |
| Unknown | 1 (3.7%) | 1 (6.7%) | 0 (0.0%) | --- |
| White | 18 (66.7%) | 10 (66.7%) | 8 (66.7%) | --- |
| Age (years) | 22 [19, 27] | 22 [19, 24] | 25 [21, 28] | 0.138 |
| Height (m) | 1.7 (0.09) | 1.6 (0.05) | 1.8 (0.05) | **<0.001** |
| Weight (kg) | 73.0 [66.6, 90.0] | 72.4 [63.5, 87.7] | 76.8 [71.1, 100.0] | 0.075 |
| BMI (kg/m2) | 26.2 [24.1, 31.4] | 26.2 [24.3, 32.0] | 24.9 [23.2, 30.4] | 0.486 |
| Fat Mass (kg) | 24.2 (9.6) | 26.3 (8.6) | 21.7 (10.6) | 0.221 |
| Fat Free Mass (kg) | 51.7 (10.9) | 45.2 (6.3) | 59.7 (10.0) | **<0.001** |
| Body Fat (%) | 30.4 (8.7) | 34.9 (6.3) | 24.7 (8.1) | **0.001** |
| VAT Volume (in3) | 22.4 [7.3, 50.8] | 11.7 [4.2, 37.5] | 30.0 [14.8, 67.1] | **0.048** |
| Resting Metabolic Rate (kcal/d) | 1710 (288) | 1538 (222) | 1924 (207) | **<0.001** |
| Respiratory Exchange Ratio (RER) | 0.80 (0.06) | 0.78 (0.05) | 0.82 (0.06) | 0.056 |
| Carbohydrate Oxidation (g/min) | 0.1 (0.07) | 0.07 (0.04) | 0.14 (0.08) | **0.005** |
| Lipid Oxidation (g/min) | 0.08 (0.02) | 0.08 (0.03) | 0.08 (0.02) | 0.910 |
| VO2PEAK (mL/kg/min) | 34.1 (8.8) | 31.4 (7.3) | 37.5 (9.7) | 0.075 |
| VO2PEAK (L/min) | 2.6 (0.6) | 2.3 (0.37) | 3.1 (0.53) | **<0.001** |
| Race/ethnicity is reported as count (percent). Numerical data are mean ± SD or median [IQR] based on normality. All data are presented using untransformed values. | | | | |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 2.** Effect of body composition on OGTT response | | | | | |  |  |  |  |
|  | **Glucose** | | | **Insulin** | | | **NEFA** | | |
| **Characteristic**1 | **Beta** | **95% CI** | **p-value** | **Beta** | **95% CI** | **p-value** | **Beta** | **95% CI** | **p-value** |
| Time2 x BF% | --- | --- | --- | --- | --- | --- | 0.080 | -0.077, 0.238 | 0.313 |
| Time x BF% | --- | --- | --- | --- | --- | --- | -0.273 | -0.399, -0.147 | **<0.001** |
| Time | -0.621 | -0.814, -0.427 | <0.001 | -0.187 | -0.323, -0.050 | **0.008** | -0.955 | -1.08, -0.830 | **<0.001** |
| Time2 | --- | --- | --- | --- | --- | --- | 0.334 | 0.177, 0.492 | **<0.001** |
| BF% | 0.802 | -0.021, 1.63 | 0.055 | 0.232 | -0.285, 0.749 | 0.355 | 0.310 | -0.345, 0.966 | 0.333 |
| Fasting Measure | 0.231 | -0.140, 0.602 | 0.206 | -0.244 | -0.859, 0.370 | 0.412 | 0.210 | -0.142, 0.563 | 0.224 |
| Sex (Male) | 0.585 | -1.02, 2.19 | 0.451 | 0.058 | -0.879, 0.995 | 0.898 | 1.330 | 0.099, 2.57 | **0.036** |
| Age | -0.098 | -0.563, 0.367 | 0.660 | 0.016 | -0.245, 0.278 | 0.897 | 0.198 | -0.149, 0.544 | 0.244 |
| Matsuda | 0.188 | -0.365, 0.741 | 0.482 | -0.596 | -1.33, 0.140 | 0.105 | 0.080 | -0.330, 0.491 | 0.683 |
| Adipo-IR (OGTT) | 0.137 | -0.393, 0.668 | 0.591 | 0.438 | 0.142, 0.735 | **0.006** | 0.797 | 0.359, 1.24 | **0.001** |
| VAT volume1/3 | -0.014 | -0.790, 0.761 | 0.969 | 0.056 | -0.378, 0.489 | 0.789 | -0.470 | -0.934, 0.001 | 0.116 |
| 1Fasting Measure denotes the fasting concentration of the outcome variable. | | | | | | |  |  |  |
| A 1-SD increase in each variable corresponds to a 1-SD increase in glucose (19.1), insulin (0.59), or NEFA (0.45). Insulin and NEFA were transformed via natural log for normality prior to analysis. Variable SDs: time: 33.697; BF%: 8.7; Fasting glucose: 8.5; Fasting insulin: 0.7; Fasting NEFA: 0.5; Age: 4.97; Matsuda: 2.9; AdipIR (OGTT): 13.3; VAT: 1.29. | | | | | | | | | |

|  |  |  |  |
| --- | --- | --- | --- |
| **Supplementary Table 3.** Effect of BF% on Postprandial Fat Oxidation (FOX) as a Measure of Metabolic Flexibility | | | |
| **Characteristic** | **Beta** | **95% CI** | **p-value** |
| Time | -0.303 | -0.401, -0.206 | **<0.001** |
| BF% | -0.334 | -0.869, 0.200 | 0.203 |
| Fasting Measure | 0.689 | 0.410, 0.968 | **<0.001** |
| Sex (Male) | -0.400 | -1.45, 0.651 | 0.431 |
| Age | 0.135 | -0.178, 0.449 | 0.374 |
| Matsuda | -0.394 | -0.769, -0.019 | **0.041** |
| Adipo-IR (OGTT) | -0.217 | -0.564, 0.130 | 0.204 |
| VAT volume1/3 | 0.457 | -0.056, 0.971 | 0.077 |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 4.** Effect of Fat Mass on Measures of Postprandial Metabolic Flexibility and Postprandial CHOX | | | | | | | | | |
|  | RER | | | CHOX | | | CHOX with FFM | | |
| **Characteristic** | **Beta** | **95% CI** | **p-value** | **Beta** | **95% CI** | **p-value** | **Beta** | **95% CI** | **p-value** |
| Time2 | -0.186 | -0.329, -0.042 | **0.012** | -0.198 | -0.347, -0.049 | **0.010** | -0.198 | -0.347, -0.049 | **0.01** |
| Time | 0.325 | 0.211, 0.439 | **<0.001** | 0.249 | 0.131, 0.368 | **<0.001** | 0.249 | 0.131, 0.368 | **<0.001** |
| Fat Mass | 0.367 | -0.303, 1.04 | 0.263 | 0.361 | -0.298, 1.02 | 0.263 | 0.362 | -0.323, 1.05 | 0.277 |
| Fasting Measure | 0.761 | 0.415, 1.11 | **<0.001** | 0.732 | 0.321, 1.14 | **0.002** | 0.721 | 0.254, 1.19 | **0.005** |
| Sex (Male) | 0.194 | -0.829, 1.22 | 0.693 | 0.309 | -0.744, 1.36 | 0.542 | 0.283 | -0.894, 1.46 | 0.616 |
| Age | -0.148 | -0.485, 0.189 | 0.365 | -0.107 | -0.442, 0.228 | 0.506 | -0.116 | -0.492, 0.260 | 0.520 |
| Matsuda | 0.449 | 0.051, 0.847 | **0.030** | 0.544 | 0.152, 0.936 | **0.010** | 0.541 | 0.131, 0.951 | **0.013** |
| AdipIR (OGTT) | 0.145 | -0.235, 0.525 | 0.430 | 0.074 | -0.304, 0.453 | 0.683 | 0.066 | -0.347, 0.480 | 0.737 |
| VAT volume1/3 | -0.468 | -1.10, 0.163 | 0.135 | -0.268 | -0.882, 0.345 | 0.367 | -0.271 | -0.910, 0.367 | 0.379 |
| Fat-free Mass | --- | --- | --- | --- | --- | --- | 0.033 | -0.503, 0.568 | 0.899 |

|  |  |  |
| --- | --- | --- |
| **Supplementary Table 5.** Confidence Intervals from Bootstrapping vs. Satterthwaite Approximation | | |
|  | RER | |
| **Characteristic** | **Satterthwaite** | **Bootstrapping** |
| BF% x Time2 | -0.308, -0.029 | -0.299, -0.056 |
| BF% x Time | -0.037, 0.185 | -0.053, 0.205 |
| BF% | 0.087, 1.22 | 0.062, 1.430 |
| Time2 | -0.336, -0.059 | -0.320, -0.063 |
| Time | 0.220, 0.440 | 0.212, 0.465 |
| Fasting Measure1 | 0.480, 1.130 | 0.450, 1.070 |
| Sex (Male) | -0.546, 1.660 | -0.705, 1.951 |
| Age | -0.413, 0.223 | -0.466, 0.247 |
| Matsuda Index | 0.126, 0.883 | 0.092, 1.203 |
| Adipo-IR (OGTT) | -0.160, 0.563 | -0.197, 0.812 |
| VAT volume1/3 | -1.02, 0.012 | -1.047, 0.162 |
| Satterthwaite CIs are from the original models. | | |

A graph with lines and dots

Description automatically generatedA graph with a number of points

Description automatically generated with medium confidence

**Supplementary Figure 1.** Metabolic flexibility and individuals with normal weight (NW) or Overweight and Obesity (OWOB) as determined by Delta RER and Delta CHO. Differences between groups (BMI classification) was compared via T-test.

A graph of a number of patients

AI-generated content may be incorrect.

**Supplementary Figure 2.** Visualization of the interaction between body fat percentage and postprandial NEFA following a 75g oral glucose tolerance test (OGTT). Visualization is comparable to **Figure 1**, except postprandial NEFA is plotted as the outcome variable. Analysis methods, justification of BF% values, and adjustment for covariates are otherwise identical.

**References**

1. Lightowler H, Schweitzer L, Theis S, Henry CJ. Changes in Weight and Substrate Oxidation in Overweight Adults Following Isomaltulose Intake During a 12-Week Weight Loss Intervention: A Randomized, Double-Blind, Controlled Trial. Nutrients. 2019;11(10). Epub 20191004. doi: 10.3390/nu11102367. PubMed PMID: 31590285; PMCID: PMC6836138.

2. Rudwill F, O'Gorman D, Lefai E, Chery I, Zahariev A, Normand S, Pagano AF, Chopard A, Damiot A, Laurens C, Hodson L, Canet-Soulas E, Heer M, Meuthen PF, Buehlmeier J, Baecker N, Meiller L, Gauquelin-Koch G, Blanc S, Simon C, Bergouignan A. Metabolic Inflexibility Is an Early Marker of Bed-Rest-Induced Glucose Intolerance Even When Fat Mass Is Stable. J Clin Endocrinol Metab. 2018;103(5):1910-20. doi: 10.1210/jc.2017-02267. PubMed PMID: 29546280; PMCID: PMC7263792.