Increased Postprandial Metabolic Flexibility is Associated with Higher Body Fat Percentages in Healthy Young Adults

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Abstract

PURPOSE: High levels of adiposity are associated with an increased risk for chronic cardiometabolic disease. Adipose tissue is a critical metabolic tissue during the postprandial (feeding) period. We sought to assess the relationship between body composition (body fat percentage) and measures of postprandial metabolic flexibility in otherwise healthy young adults. **METHODS:** Young adults (n = 27, n = 15 females) of varying body compositions (BMI = 27.1 ± 4.5; Body Fat (BF)% = 30.4 ± 8.7) but without overt pathology completed a 100g oral glucose tolerance test (OGTT). Indirect calorimetry was conducted before (fasting) and following (30, 60, 90, 120 min) consumption of the glucose beverage. Serum and plasma were collected at corresponding time points and analyzed for blood glucose, insulin, non-esterified fatty acids, and inflammatory markers. Linear mixed models were used to test for the effect of body composition on postprandial metabolism over time while controlling for sex, age, fasting metabolism, measures of insulin sensitivity, visceral adipose tissue volume, and the repeated measures design. **RESULTS:** As expected, there was a significant ($p < 0.05$) increase in blood glucose, serum insulin, RER, and CHO with concomitant decreases in plasma NEFAs and whole-body FOX during the OGTT. Using mixed effects models, BF% modified the relationship between postprandial RER and time (β = -0.133, p = 0.019), as well as postprandial CHO and time (β = -0.134, p = 0.023) such that individuals with higher BF% increase their RER and CHO at a greater rate and to a greater extent than those with lower BF%. There was no evidence that BF% modifies the relationship between FOX and time. **CONCLUSION:** We demonstrate that a higher body fat percentage is associated with greater postprandial metabolic flexibility (RER and CHO) during an OGTT in young adults without overt metabolic pathology. Metabolic flexibility in this sample may be preserved and represents a compensatory adaptation to decreased glucose storage in the anabolic, postprandial period.

Introduction

Metabolic flexibility is described as the appropriate shift in substrate oxidation based upon substrate availability (e.g. fasting to fed) or metabolic demand (e.g. exercise). It was originally described as changes in the respiratory quotient (RQ) across the leg limb from rest to insulin stimulation during the euglycemic-hyper-insulinemic clamp (EHC).[1, 2] In the two decades since the concept of metabolic flexibility was described in humans, several methodological approaches to assess metabolic flexibility have been utilized, including indirect calorimetry during the EHC, during oral glucose tolerance tests (OGTT), and during mixed-meal feeding challenges (MMC).[1, 3] Individuals with overt metabolic pathologies, such as type 2 diabetes and insulin resistance, as well as individuals classified as obese based on BMI, have been described as metabolically inflexible.[1] That is, these individuals showed blunted (reduced) changes in the respiratory exchange ratio (RER) during metabolic challenges such as EHC and feeding.

In the absence of overt metabolic pathology or metabolic dysfunction, transitioning from the fasted to the fed state involves shifting away from aerobic fatty acid oxidation to aerobic glucose oxidation and, to a lesser degree, glycolytic energy production.[4] This shift results in an anabolic environment, in which exogenous energy is stored, as compared to the catabolic environment of fasting. Elevated insulin, which is the primary driver of the metabolic shift following feeding, suppresses adipose tissue lipolysis and promotes the uptake, storage, and oxidation of exogenous macronutrients.[4] Adipose tissue serves a critical role in buffering fatty acid flux in the circulation during this postprandial period; it is also a site of significant glucose uptake for lipogenesis and has been identified as a central regulator of metabolic flexibility.[2, 4]

The presence of obesity and increased fat mass is believed to negatively impact metabolic flexibility.[5, 6] Individuals with obesity have higher resting RER indicating a preference for higher carbohydrate oxidation in the fasting state while adipose tissue lipolysis is more resistant to insulin-induced suppression in feeding. However, studies have not thoroughly

analyzed the effect of adiposity on postprandial metabolic flexibility. By dividing participants into high vs. low metabolic flexibility, Sparks et al. (2009) demonstrated that healthy young males with low metabolic flexibility during insulin stimulation (EHC) had significantly higher body fat percentage and larger fat cell size.[6] Follow-up analysis in this cohort demonstrated that females were more metabolically flexible than their male counterparts despite significantly higher body fat percentages.[5] Conversely, individuals classified as having high metabolic flexibility to an OGTT had similar body fat percentages but a larger subcutaneous adipose tissue (SAT) as compared to those described as having low metabolic flexibility.[7] This discrepancy was highlighted in a recent systematic review by Glaves et al. (2021), who found inconsistent and unclear relationships between adipose tissue volume, function, and characteristics in studies using EHC, OGTTs, and MMCs.[3]

The primary objective of this study was to thoroughly assess the relationship between adiposity and postprandial metabolic flexibility during an OGTT. We recruited 27 healthy male and female young adults (18 - 40 years old) with body mass index (BMI) between 18.5-39.9. Using indirect calorimetry, we assessed postprandial metabolic flexibility (RER, Carbohydrate Oxidation [CHO], and Fat Oxidation [FOX]) during an OGTT. We hypothesized that individuals with higher body fat percentages would demonstrate significantly reduced postprandial metabolic flexibility (respiratory exchange ratio and carbohydrate oxidation) throughout the OGTT.

METHODS

Participants

We performed a cross-sectional, experimental study from January 2023 to January 2024. Potential participants were recruited and screened based on sex, body mass index (BMI) classification, and lack of known clinical pathology from the George Washington University campus and surrounding metropolitan area. Participants were required to be between 18 and 40 years old, have a BMI between 18.5 and 39.9, be free of clinically diagnosed cardiometabolic

disease, and not be on prescription medications for hypertension, blood lipids, or blood glucose homeostasis. Participants who were pregnant or taking medications for cardiometabolic disease were excluded. Recruitment was stratified by BMI classification to yield a across the BMI continium. In response to flyers and emails, fifty-two participants were screened for participation. Of these, thirty-four were enrolled in this study; seven participants did not complete the study (3 due to scheduling, 3 due to loss to follow-up, 1 due to equipment malfunction), giving complete data for 27 participants. Participant characteristics are reported in Table 1. All study procedures were approved by the George Washington University IRB (IRB #202737). Prior to enrollment, participants read and signed an IRB-approved informed consent and all study procedures were conducted in accordance with the Declaration of Helsinki.

Experimental Design

Participants reported to the Metabolic and Exercise Testing Laboratories in the morning between 0800 and 0900 on two separate days. Each visit was separated by at least 48 hours. At the first visit, participants completed written questionnaires for medical and physical activity history, assessment of body composition, and cardiorespiratory fitness. Participants returned to the lab for assessment of postprandial substrate metabolism following an OGTT.

For each visit, participants were instructed to arrive after 10 hours of fasting and to abstain from caffeine consumption for 12 hours and from any recreational drug use or structured exercise for 24 hours. Upon arrival, hydration was measured using a portable refractometer. If urine specific gravity was above 1.02, participants were given eight ounces of water. Body weight was measured using a mechanical column scale (Seca 700; Hamburg, Germany).

Table 1. Participant Anthropometric Characteristics

Variable	All (n = 27)	Female (n = 15)	Male (n = 12)	p-value (Sex)	Association with BF%
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	r=0.081; p=0.688
Height (m)	1.7 (0.09)	1.6 (0.05)	1.8 (0.05)	**<0.001**	**r=-0.415; p=0.032**
Weight (kg)	73.0 [66.6, 90.0]	72.4 [63.5, 87.7]	76.8 [71.1, 100.0]	0.075	r=0.257; p=0.195
BMI (kg/m^2)	27.1 (4.5)	27.7 (4.7)	26.4 (4.4)	0.474	**r=0.582; p=0.001**
Fat Mass (kg)	24.2 (9.6)	26.3 (8.6)	21.7 (10.6)	0.221	**r=0.854; p<0.001**
Fat Free Mass (kg)	51.7 (10.9)	45.2 (6.3)	59.7 (10)	**<0.001**	r=-0.351; p=0.072
Body Fat (%)	30.4 (8.7)	34.9 (6.3)	24.7 (8.1)	**0.001**	---
VAT Volume (in^3)	2.7 (1.3)	2.2 (1.3)	3.3 (1.0)	**0.035**	r=0.301; p=0.127
RMR (kcal/d)	1709.8 (287.9)	1538.2 (222.3)	1924.2 (206.8)	**<0.001**	r=-0.25; p=0.208
RER	0.80 (0.06)	0.78 (0.05)	0.82 (0.06)	0.056	r=-0.129; p=0.520
Carbohydrate Oxidation (g/min)	0.10 (0.07)	0.07 (0.04)	0.14 (0.08)	**0.005**	r=-0.16; p=0.424
Lipid Oxidation (g/min)	0.08 (0.02)	0.08 (0.03)	0.08 (0.02)	0.91	r=-0.031; p=0.878
VO_{2PEAK} (mL/kg/min)	34.1 (8.8)	31.4 (7.3)	37.5 (9.7)	0.075	**r=-0.784; p<0.001**
VO_{2PEAK} (L/min)	2.6 (0.6)	2.3 (0.37)	3.1 (0.53)	**<0.001**	**r=-0.66; p<0.001**

Race/ethnicity is reported as count (percent). Numerical data are mean (SD) or median [IQR] based on normality. RMR = Resting metabolic rate, RER = Respiratory Exchange Ratio

Body mass and body composition

Height was measured using a wall-mounted stadiometer (Seca 216; Hamburg, Germany) to the nearest 0.1 centimeters. Weight was measured using the digital scale as part of the bioelectrical impedance analysis (InBody 770; Seoul, South Korea). Dual X-ray absorptiometry (GE Lunar iDXA; GE Healthcare, Chicago, IL, USA) with manufacturer software (enCORE Forma version 15) was used to measure body fat percentage, fat-free mass (FFM), fat mass, and visceral adipose tissue (VAT) volume. Before the measurement, participants removed all jewelry and were instructed to wear clothing free of metal zippers or reflective surfaces.

Exercise Testing

Following body composition assessment, participants completed a graded exercise test on a cycle ergometer (Monark LC6 novo; Varberg, Sweden) to volitional exhaustion. 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. The test continued until the participant was unable to continue cycling or the investigator observed a plateau in oxygen consumption (VO_2). Breath-by-breath data were averaged into five-second bins. Because not every participant satisfied the criteria for VO2max,[8] the highest 15-second value is reported as VO2peak.

Oral Glucose Tolerance Test with Indirect Calorimetry and Blood Sampling

On a separate day, participants arrived following the same pre-testing guidelines outlined above. Assessment of body weight and hydration were performed, and the participant rested in a reclined chair in a dimly lit room for at least 10-15 minutes. A venous catheter was placed in an antecubital vein and fasting whole blood was sampled for plasma (K-EDTA) and serum (Serum Separator Tube); saline was used to keep the catheter patent between blood draws. After the resting blood draw, indirect calorimetry was conducted via the canopy method (Parvo Medics TrueOne 2400; Salt Lake City, Utah) while the participant lay supine on a padded table for determination of fasting metabolic measures. Following this, subjects consumed a 100g glucose beverage (GlucoCrush Cardinal Health; Dublin, OH) and were instructed to finish consumption of the drink within 5 minutes. After finishing the drink, subjects returned to the supine position for the remainder of the test. Indirect calorimetry was used to assess postprandial substrate oxidation at the following postprandial intervals (min): 15 – 30, 45 – 60, 75 – 90, 105 – 120.

Determination of substrate oxidation and metabolic rate

For each 15-minute indirect calorimetry measurement, expired gases were collected from a mixing chamber at 5-second intervals, and the final 8 minutes were used for analysis. Respiratory exchange ratio (RER) was calculated as the ratio between exhaled carbon dioxide (VCO_2) and VO_2. Metabolic rate and rates of carbohydrate (CHO) and fat oxidation (FOX) were calculated using published equations for fat oxidation = [VO_2 (L/min) x 1.67) – (VCO_2 (L/min) x

127 1.67)] and carbohydrate oxidation = [VCO_2 (L/min) x 4.55) – (VO_2 (L/min) x 3.21)].[9] For
128 metabolic rate calculations, urinary nitrogen excretion was assumed to be zero.

129 *Laboratory measurements and calculations*

130 Fasting and postprandial blood glucose were determined in fresh whole blood
131 (OneTouch Ultra, Lifescan, Inc.; Milpitas, CA). Fasting and postprandial serum samples were
132 used to measure insulin (Insulin Human ELISA; Invitrogen Inc. CA), and non-esterified fatty
133 acids (NEFA; LabAssay NEFA; FujiFilm; Richmond, VA). Fasting serum samples were used to
134 determine C-reactive protein (CRP; CRP Human ELISA; Invitrogen Inc., CA), leptin (Leptin
135 Human ELISA; Invitrogen Inc., CA), and adiponectin (Adiponectin human ELISA; Adipogen Life
136 Science; San Diego, CA). Manufacturer instructions were followed for conducting and analyzing
137 all assays. Manufacturer internal controls were assessed along with coefficients of variations for
138 biological replicates. Samples whose performance was assessed to be outside of manufacturer
139 recommendations were re-analyzed. Fresh whole blood (fasting) was used to determine
140 HbA1C% (PTS Diagnostics; Whitestown, IN) and blood lipid profiles (CardioChek; PTS
141 Diagnostics; Whitestown, IN) via point-of-care analyzers

142 Matsuda Index was calculated as follows: 10,000/√ [fasting glucose (mg/dl) × fasting
143 insulin (μIU/mL)] × [mean glucose (mg/dl) × mean insulin (μIU/mL) during OGTT before
144 normalizing to the 100g dose.[10] Adipose tissue insulin resistance (Adip-IR) was calculated using
145 fasting values ADIP-IR (fasting) = fasting insulin (μIU/mL) * fasting NEFA (mEq/L) and
146 postprandial (30, 60, 90, 120) values ADIP-IR (OGTT) = mean plasma NEFA (mEq/L) x mean
147 serum insulin (μIU/mL).[11] Fasting insulin and glucose measurements were used to calculate the
148 homeostatic model assessment of insulin resistance (HOMA-IR) = [fasting insulin (μIU/mL) -
149 (fasting glucose (mg/dl)/18.156)] / 22.5.[12]

150 *Statistical Analyses*
151
152 We report mean (sd) or median (interquartile range, IQR) both overall and by sex for
153 anthropometric (**Table 1**) and clinical (**Table 2**) measures and report frequency (percent) for

154 race. We conducted two-sample t-tests or Mann-Whitney tests to compare numerical variables
155 between the sexes for normally and non-normally distributed variables, respectively. We
156 compared race categories between the sexes using Fisher's exact test. We computed and
157 tested the association between each of the clinical variables and percent body fat (BF%) using
158 Pearson correlation coefficients. To describe the change in each postprandial outcome variable
159 during the OGTT (i.e., RER, CHO, FOX, glucose, insulin, and NEFA), we used one-way,
160 repeated measures analysis of variance (ANOVA) with time modeled as a categorical variable.
161 Contrasts were set *a priori* as the comparison from baseline to each postprandial timepoint (i.e.,
162 0 vs. 30, 0 vs. 60, etc.) with a Sidak adjustment for multiple comparisons. Separately, linear
163 mixed-effects models were used to determine the effect of body composition on postprandial
164 metabolism. For each postprandial outcome variable, we included fixed effects for time as a
165 continuous variable, percent body fat (BF%), time x BF% interaction, sex, age, Matsuda index,
166 AdipIR, visceral adipose tissue volume, and the fasted outcome measure. A subject-specific
167 random intercept term was included to account for repeated measures. To account for the
168 possible nonlinear effect of time on postprandial metabolism (i.e., a nonlinear change in
169 metabolism), models with an additional quadratic time term and quadratic time x BF% were
170 considered. Models with and without the quadratic time terms were compared using likelihood
171 ratio tests to determine if the quadratic terms should be retained. Variables in the models were
172 tested for normality with the Shapiro-Wilk test and transformed. As a result, insulin and NEFA
173 concentrations were transformed via natural log, and VAT volume was transformed via cube
174 root. Each variable included in the models was standardized to a mean of 0 and standard
175 deviation of 1, and time was divided by 30 to correct scaling. Fasting and postprandial variables
176 were standardized separately due to the inclusion of the fasting state as a covariate in the
177 models. All analyses were conducted in R (version 4.2.2). The lmerTest[13] and performance[14]
178 packages were used for model fitting and checking of relevant assumptions, and the emmeans
179 package[15] was used to visualize continuous interactions. For the interpretation of linear mixed-

effects models, we assess significance using both the 95% confidence interval (e.g., does it cross 0) and the p-value ($p < 0.05$). Due to ambiguity in the determination of p-values for these models[16], we deferred to the confidence interval when there was disagreement between the confidence intervals and p-values.

RESULTS

There were 27 young adults (15 (55.6%) female) with no previously diagnosed metabolic pathology. Body composition was heterogeneous across the sample, as indicated by the wide interquartile range, suggesting our sample is sufficient to assess the relationship between body composition and postprandial metabolic flexibility. Compared to females, males had higher resting carbohydrate oxidation ($p = 0.005$) and resting metabolic rate ($p < 0.001$) without differences in resting lipid oxidation (**Table 1**). Absolute VO_{2PEAK} was higher ($p <0.001$) in males than females with no significant differences in relative VO_{2PEAK} ($p = 0.075$). Triglycerides were higher in males than females ($p = 0.026$), and leptin was higher in females than males ($p<0.001$) (**Table 2**). All other analyzed clinical measures were similar between the sexes.

Table 2. Participant Clinical Data

Variable	All (n = 27)	Female (n = 15)	Male (n = 12)	p-value (Sex)	Association with BF%
Blood Glucose (mg/dL)	100 (8)	101 (10)	99 (7)	0.580	r=0.036; p=0.858
Serum Insulin (µIU/mL)	2.5 (0.68)	2.6 (0.56)	2.2 (0.79)	0.173	**r=0.427; p=0.037**
NEFA (mEq/L)	-1.01 (0.52)	-0.93 (0.6)	-1.12 (0.4)	0.383	r=0.371; p=0.068
HbA1c (%)	5.1 [4.8, 5.25]	5.0 [4.8, 5.2]	5.15 [5.03, 5.3]	0.432	r=-0.221; p=0.269
Total Cholesterol (mg/dL)	160.4 (33.9)	156.2 (30.1)	166.2 (39.5)	0.489	r=0.198; p=0.353
HDL (mg/dL)	51.0 [47.8, 59.5]	50.0 [45.2, 67.0]	51.5 [50.0, 56.5]	0.725	r=0.086; p=0.691
LDL (mg/dL)	92.3 (29.6)	88.6 (22.7)	97.3 (37.9)	0.517	r=0.085; p=0.715
Triglycerides (mg/dL)	74.5 [66.2, 93.0]	70.5 [64.8, 76.0]	94.5 [77.2, 119.5]	**0.026**	r=-0.016; p=0.940
Matsuda Index	4.24 [2.58, 6.00]	3.00 [2.48, 4.70]	5.96 [4.14, 6.67]	0.074	**r=-0.616; p=0.001**
HOMA-IR	3.4 (2.0)	3.8 (1.8)	2.9 (2.3)	0.277	r=0.398; p=0.054
AdipIR (OGTT)	7.7 [4.4, 12.2]	8.8 [6.2, 13.8]	6.5 [3.5, 8.8]	0.259	**r=0.481; p=0.017**
AdipIR (fasting)	5.2 [3.0, 6.9]	5.8 [5.2, 12.4]	3.4 [2.4, 4.0]	0.056	**r=0.443; p=0.030**
Adiponectin (ng/mL)	4613 [3955, 6013]	4798 [4145, 6847]	4175 [3639, 5433]	0.291	r=-0.151; p=0.470
Leptin (pg/mL)	118.1 [53.8, 148.5]	143.5 [114.7, 254.0]	45.2 [30.6, 88.1]	**<0.001**	**r=0.817; p<0.001**
CRP (mg/L)	0.48 [0.26, 1.20]	0.36 [0.27, 0.68]	0.64 [0.32, 1.60]	0.291	r=0.091; p=0.664

Data are mean (SD) or median [IQR], depending on normality. VAT volume was adjusted with a cube root transformation. Insulin and NEFA concentrations were transformed via natural log.

198 *Substrate Response to the OGTT*

199 Individual and aggregate data from the OGTT is presented by sex for outcome
200 measures in the blood (**Figure 1**) and whole-body substrate oxidation (**Figure 2**); all aggregate
201 values are available in **Supplementary Table 1**. During the OGTT, one-way repeated
202 measures ANOVAs indicated increases in blood glucose, serum insulin, RER, and CHO with
203 concomitant decreases in plasma NEFAs and whole-body FOX. Blood glucose was elevated
204 above fasting levels (99.7 ± 8.5 mg/dL) at 30 (139.9 ± 21.0; $p<0.001$), 60 (124.9 ± 29.6;
205 $p<0.001$), and 90 (115.3 ± 25.5; $p = 0.007$) minutes but not 120 minutes (109 ± 21.7; $p = 0.214$).
206 Compared to fasting insulin (14.2 ± 8.1 μIU/mL), postprandial insulin was elevated at 30 (79.9 ±
207 75.1), 60 (77.9 ± 54.2), 90 (63.2 ± 47.7), and 120 (55.0 ± 36.4) minutes (all $p<0.001$). NEFAs
208 were suppressed compared to fasting (0.41 ± 0.19 mEq/L) at 30 (0.28 ± 0.13), 60 (0.15 ± 0.08),
209 90 (0.15 ± 0.08), and 120 (0.09 ± 0.04) minutes (all $p<0.001$). RER was not different from
210 fasting (0.795 ± 0.055) at 30 minutes (0.806 ± 0.052; $p = 0.493$) but was significantly higher at
211 60 (0.832 ± 0.051; $p<0.001$), 90 (0.839 ± 0.044; $p<0.001$), and 120 (0.845 ± 0.048; $p<0.001$)
212 minutes. Similarly, CHO was not different from fasting (0.102 ± 0.071 g/min) at 30 minutes
213 (0.125 ± 0.071; $p = 0.083$) but was significantly higher at 60 (0.157 ± 0.063; $p<0.001$), 90 (0.160
214 ± 0.049; $p<0.001$), and 120 (0.166 ± 0.061; $p<0.001$) minutes. FOX was unchanged from fasting
215 (0.084 ± 0.024) at 30 (0.086 ± 0.024; $p = 0.927$) and 60 (0.078 ± 0.027; $p = 0.383$) minutes. At
216 90 (0.073 ± 0.026; $p = 0.011$) and 120 minutes (0.068 ± 0.023; $p<0.001$), FOX was suppressed
217 below fasting levels.

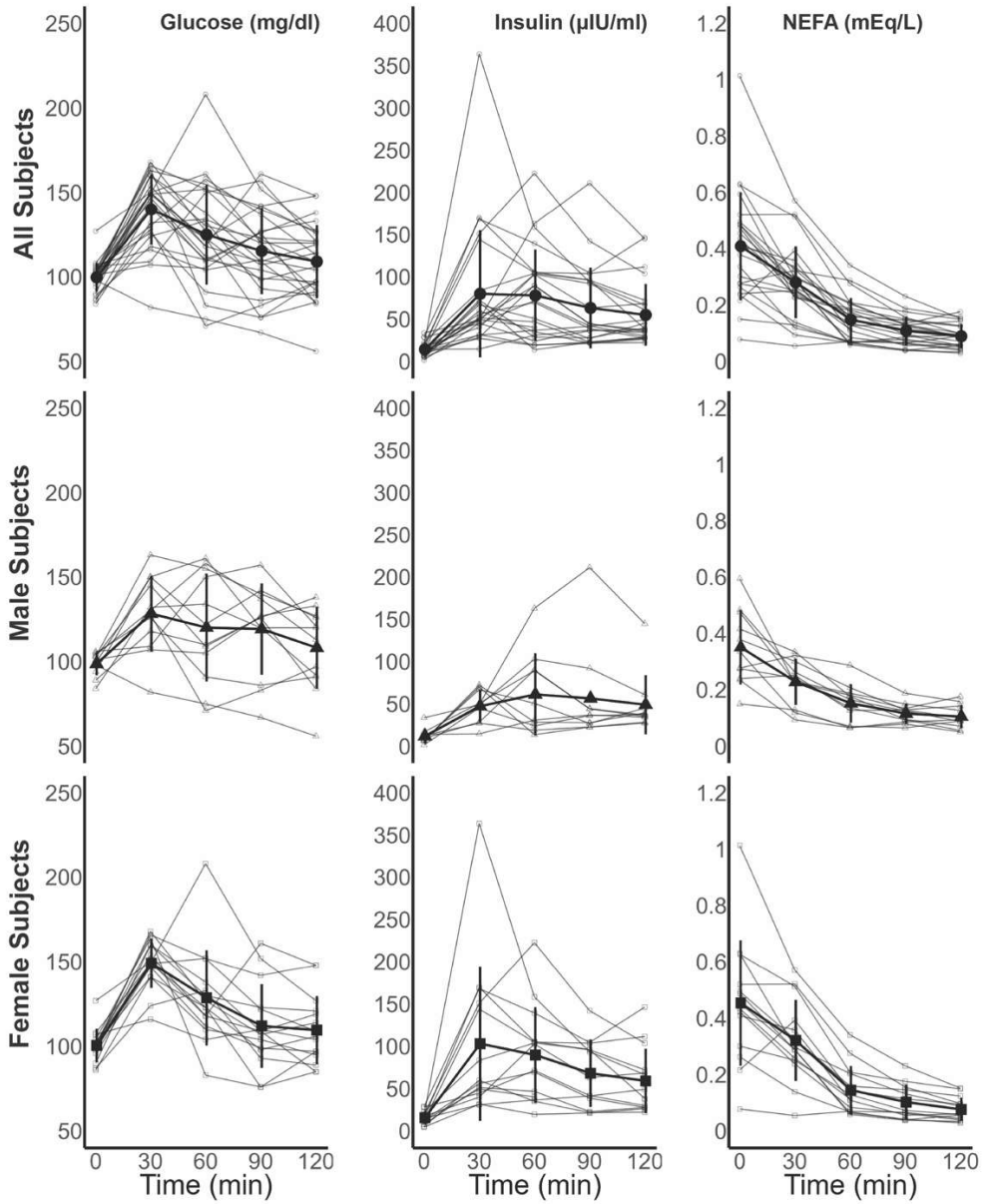

Figure 1. Substrate (Glucose: Left column; NEFA: right column) and hormonal (Insulin: middle column) response to a 100g oral glucose tolerance test in young adults (Top; All Subjects, Middle; Males, Bottom; Females) with no overt metabolic pathology. Black lines are mean (SD) while lighter grey lines are individual responses. Data for the means and SDs of each postprandial measure is supplied in Supplementary Table 1.

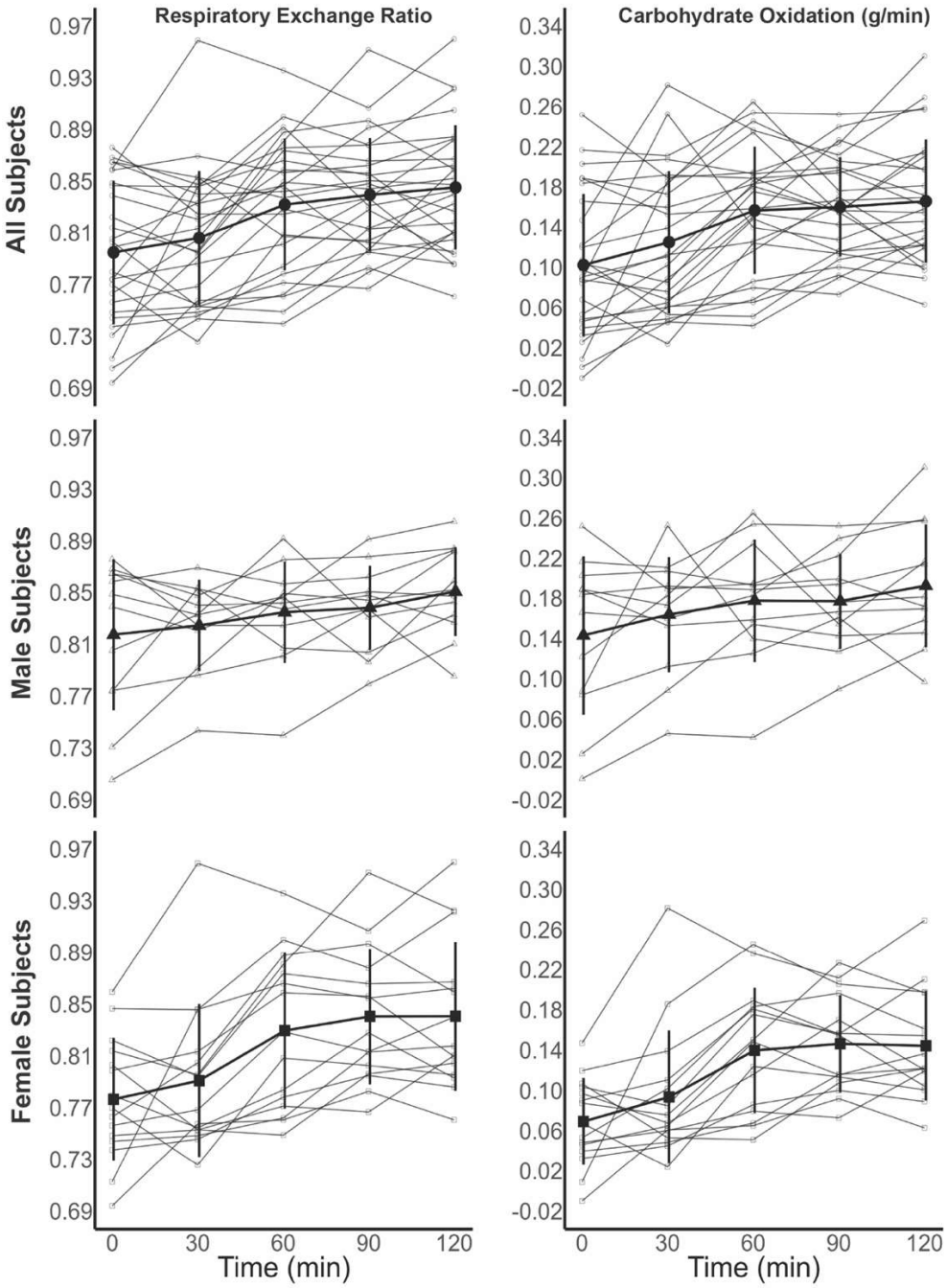

Figure 2. Metabolic response (Respiratory Exchange Ratio: left column; Carbohydrate Oxidation: Right column) measured by Indirect calorimetry, to a 100g oral glucose tolerance test in young adults (Top; All Subjects, Middle; Males, Bottom; Females) with no overt metabolic pathology. Black lines are mean (SD) while lighter grey lines are individual responses. Data for the means and SDs of each measure is supplied in Supplementary Table 1.

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 time^2 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 3**). The addition of a time^2 main effect and a time^2 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 time^2, 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 6.9% increase in body fat percentage would result in a significant ($\beta = 0.802$; 95% CI: 0.155, 1.45; $p = 0.055$) 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 ($\beta = 0.232$; 95% CI: -0.175, 0.638; $p = 0.355$). For NEFAs, model fit was improved by the addition of a time^2 main effect and a time^2 x BF% interaction ($p < 0.001$) compared to the model with only linear time terms. In this quadratic model, BF% did not modify the relationship between NEFAs and time ($p = 0.082$) or time^2 ($p = 0.313$). In the main effects model without the interaction term, a 3.4% body fat percent increase resulted in a non-significant ($\beta = 0.39$; 95% CI: -0.115, 0.894; $p = 0.216$) increase postprandial plasma NEFA 1.19 (mEq/L). Thus, body composition affects postprandial blood glucose but not serum insulin or NEFAs throughout the OGTT (**Table 3**).

Table 3. Effect of body composition on glucose, insulin, and NEFAs during an OGTT

	Glucose			Insulin			NEFA		
Characteristic[1]	**Beta**[2]	**95% CI**	**p-value**	**Beta**	**95% CI**	**p-value**	**Beta**	**95% CI**	**p-value**
Time	-0.55	-0.723, -0.382	**<0.001**	-0.17	-0.286, -0.046	**0.008**	-2.13	-2.83, -1.44	**<0.001**
Time^2	---	---	---	---	---	---	0.26	0.123, 0.397	**<0.001**
BF%	0.802	0.155, 1.45	0.055	0.232	-0.175, 0.638	0.355	0.39	-0.115, 0.894	0.216
Fasting Measure	0.231	-0.061, 0.523	0.206	-0.24	-0.728, 0.239	0.412	0.211	-0.067, 0.488	0.224
Sex (Male)	0.585	-0.676, 1.84	0.451	0.058	-0.681, 0.794	0.898	1.33	0.360, 2.31	**0.036**
Age	-0.1	-0.464, 0.267	0.66	0.016	-0.190, 0.224	0.897	0.198	-0.075, 0.472	0.244
Matsuda Index	0.188	-0.247, 0.623	0.482	-0.6	-1.17, -0.018	0.105	0.08	-0.242, 0.403	0.683
AdipIR (OGTT)	0.137	-0.280, 0.554	0.591	0.438	0.205, 0.671	**0.006**	0.797	0.452, 1.14	**0.001**
VAT volume$^{1/3}$	-0.01	-0.624, 0.595	0.969	0.056	-0.285, 0.396	0.789	-0.47	-0.934, 0.001	0.116

[1]Fasting Measure denotes the fasting concentration of the outcome variable.
[2]A one-unit increase in time represents 30 minutes. 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. Variable SDs: 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.

Effect of body composition on postprandial metabolic flexibility (RER, CHO, and FOX)

We used mixed effects models to test for an effect of BF% on postprandial metabolic flexibility (**Table 4**). In these models, a quadratic model with a main effect for time^2 and a time^2 x BF% interaction term was a better fit to the postprandial data for RER (p = 0.002) and CHO (p = 0.002) than the linear model. In the quadratic models, BF% modified the relationship between postprandial RER and time (p = 0.011) and time^2 (p = 0.019), as well as postprandial CHO and time (p = 0.014) and time^2 (p = 0.023), indicating a nonlinear change in RER and CHO throughout the OGTT that depends on body composition. There was no evidence that BF% modifies the relationship between FOX and time (**Supplementary Table 2 and Supplementary Figure 1**). We also considered the effect of fat mass on postprandial RER and CHO. However, there was no evidence that fat mass modifies the relationship between postprandial RER and time (p = 0.364) or time^2 (p = 0.409), as well as postprandial CHO and time (p = 0.364) or time^2 (p = 0.409; see **Supplementary Table 3)**.

To assess this interaction of time and BF% as a measure of postprandial metabolic flexibility, we visualized the effect of time and BF% as continuous variables. We display (**Figure 3**) the modeled RER and CHO for three representative values of BF%: the mean BF%, the mean BF% + 1 SD, and the mean BF% - 1 SD. The interaction of time and BF% displayed in these plots indicates that higher BF% is associated with a greater rate of increase in

postprandial RER (**Figure 3a**) and CHO (**Figure 3b**). This demonstrates that higher BF% is associated with greater postprandial metabolic flexibility in our sample of young adults. The resulting visualization is adjusted for all covariates in **Table 4**.

Further, greater insulin sensitivity (Matsuda Index) was associated with a higher postprandial RER or CHO (**Table 4**). Because skeletal muscle is a primary site of postprandial glucose disposal[17] and people with obesity often have higher fat-free mass,[18] 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 time^2 main effect and a time^2 x BF% interaction term improved the model fit (p = 0.002), and percent body fat modified the relationship between CHO and time (p = 0.014) and time^2 (p = 0.023) (**Table 4**). Like RER and CHO, higher BF% was associated with greater CHO adjusted for FFM (**Figure 3c**), indicating FFM does not affect the interaction between time and BF%.

Table 4. Effect of body composition (body fat percentage) on postprandial metabolic flexibility

	RER			**CHO (g/min)**			**CHO (g/min/FFM)**		
Characteristic[1]	**Beta**[2]	**95% CI**	**p-value**	**Beta**	**95% CI**	**p-value**	**Beta**	**95% CI**	**p-value**
BF% x Time^2	-0.133	-0.240, -0.026	**0.019**	-0.134	-0.245, -0.023	**0.023**	-0.134	-0.245, -0.023	**0.023**
BF% x Time	0.732	0.189, 1.28	**0.011**	0.74	0.175, 1.31	**0.014**	0.74	0.175, 1.31	**0.014**
BF%	-0.346	-1.07, 0.374	0.39	-0.51	-1.26, 0.239	0.225	-0.311	-1.11, 0.492	0.502
Time^2	-0.156	-0.263, -0.050	**0.006**	-0.166	-0.277, -0.056	**0.005**	-0.166	-0.277, -0.056	**0.005**
Time	1.08	0.537, 1.61	**<0.001**	1.06	0.498, 1.62	**<0.001**	1.06	0.498, 1.62	**<0.001**
Fasting Measure	0.803	0.549, 1.06	**<0.001**	0.793	0.474, 1.11	**<0.001**	0.7	0.358, 1.04	**0.005**
Sex (Male)	0.556	-0.310, 1.42	0.3	0.421	-0.487, 1.33	0.451	0.458	-0.422, 1.34	0.414
Age	-0.095	-0.345, 0.155	0.536	-0.07	-0.332, 0.192	0.661	-0.139	-0.414, 0.137	0.429
Matsuda Index	0.505	0.207, 0.802	**0.012**	0.558	0.246, 0.869	**0.009**	0.59	0.285, 0.895	**0.007**
AdipIR (OGTT)	0.201	-0.083, 0.486	0.255	0.123	-0.176, 0.421	0.504	0.069	-0.232, 0.370	0.715
VAT volume$^{1/3}$	-0.504	-0.909, -0.098	0.055	-0.224	-0.648, 0.199	0.39	-0.368	-0.837, 0.100	0.224
FFM	---	---	---	---	---	---	0.305	-0.177, 0.786	0.323

[1]Fasting Measure denotes the fasting measurement of the outcome variable. See main text for other abbreviations.

[2]A one-unit increase in time represents 30 minutes. A 1-SD increase in each covariate corresponds to a 1-SD increase in postprandial RER (0.044) or CHO (0.055 g/min). Covariate SDs: Fasting RER: 0.055; Fasting CHO: 0.071; FFM: 10.9; See Table 3 for other relevant SDs.

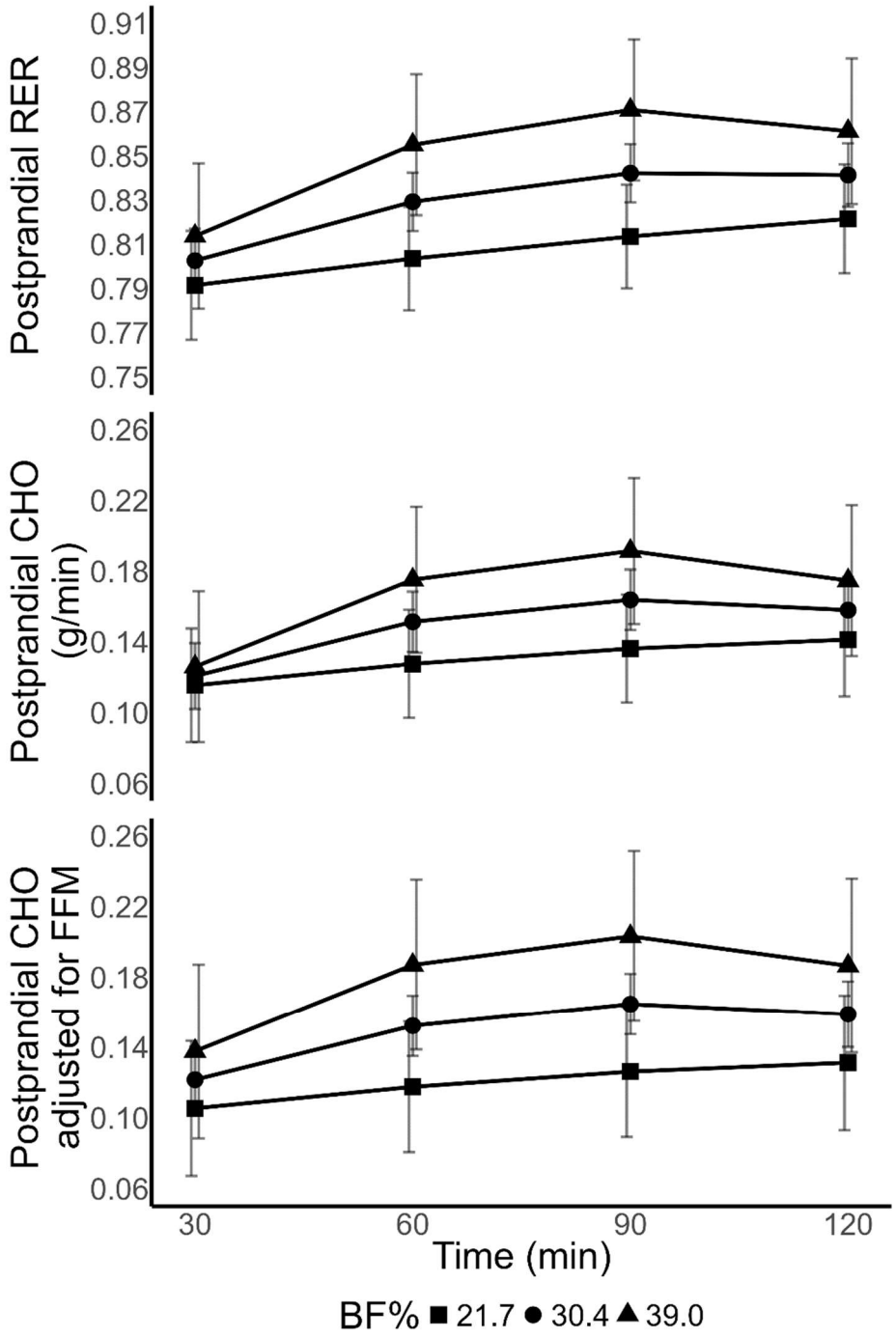

Figure 3. Visualization of the interaction between body fat percentage and postprandial metabolic metabolism as a measure of metabolic flexibility. Respiratory Exchange Ratio (RER) and carbohydrate oxidation (CHO) were plotted as continuous variables across time for representative body fat percentages (BF%) from our sample. The mean BF% of our sample was 30.4 (circles) while 21.7 (squares) and 39.0 (triangles) represent the mean ± 1 SD of BF% in our sample. Error bars represent the 95% confidence interval for each timepoint estimate for a given BF%. The data presented represents modeled data from the interaction of BF% x Time and BF% x$Time^2$ adjusted for covariates.

302 **DISCUSSION**

303 Metabolic flexibility is the ability to adjust substrate oxidation based on substrate
304 availability or demand.[1] The relationship between adiposity and measures of metabolic
305 flexibility has been poorly analyzed and lacks clarity.[3, 19-24] The majority of studies to
306 date have categorized individuals based on BMI (an indicator of adiposity) and made
307 weak, indirect associations between adiposity and measures of metabolic flexibility.[3] In
308 this analysis, we analyzed the interaction between body fat percentage and postprandial
309 metabolism (RER, CHO, and FOX) and demonstrate that higher body fat percentage is
310 associated with elevated postprandial metabolic flexibility (RER and CHO) during an
311 OGTT in young adults without overt metabolic pathology. These findings may seem
312 paradoxical since individuals with higher BMI (e.g., obesity) are most often reported to
313 have lower, or blunted, metabolic flexibility. However, this finding is not inconsistent with
314 studies that show females have greater metabolic flexibility than males despite higher
315 body fat[5] and individuals with high metabolic flexibility had greater subcutaneous
316 adipose tissue volumes.[7] Furthermore, loss of subcutaneous adipose tissue during
317 prolonged bed rest is accompanied by decreased metabolic flexibility.[20] Thus, our work
318 expands on previously published reports by directly analyzes the relationship between
319 body fat and postprandial metabolic flexibility while adjusting for known covariates that
320 significantly affect substrate oxidation.

321 Adipose tissue function is proposed to be central to the regulation of metabolic
322 flexibility[2] by buffering fatty acid flux during the postprandial period.[4] The obese state is
323 associated with increased adipose tissue volume,[25] elevated adipose tissue insulin
324 resistance, adipose tissue inflammation, and altered adipokine release;[26] all of which

325 are associated with altered postprandial metabolism.[27] In our healthy young adults,
326 higher body fat percentages did not alter suppression of lipolysis, as measured by
327 plasma NEFAs, following ingestion of the glucose beverage. Furthermore, measures of
328 adipose tissue insulin resistance (ADIP-IR) did not have a significant effect on
329 postprandial RER or CHO. However, we cannot fully rule out reduced adipose tissue
330 function in our findings. A critical role of adipose tissue in the postprandial state is
331 glucose uptake and lipogenesis which cannot be accounted for here.[28-30] Importantly,
332 our approach demonstrates that an increase in body fat percentage of ~6.9% (i.e., 20%
333 to 26.9%) would result in an increase in approximately 19.1 mg/dl of blood glucose at
334 each time point during the OGTT (**Table 3**). This suggests the capacity for adipose
335 tissue to buffer the blood glucose flux over time may be reduced in people with a higher
336 body fat percentage, which would be consistent with observational studies in individuals
337 with obesity, diabetes, and animal models.[31-34] This function is an important part of
338 adipose tissue's role in reducing hyperglycemia in the postprandial period while
339 subsequently storing nutrients for later oxidation.

340 In the context of greater hyperglycemia following the OGTT, the elevated
341 postprandial metabolic flexibility (postprandial RER and CHO) associated with higher
342 body fat percentage in our sample would not be paradoxical. The postprandial period
343 should induce an anabolic state (e.g. storage of nutrients) as compared to the catabolic
344 state of fasting (e.g. breakdown of substrate).[4] Metabolic flexibility is an critical
345 metabolic adaptation to handle the complex integration of metabolism during caloric
346 excess and restriction.[35] Furthermore, it is essential for regular maintenance of blood
347 glucose homeostasis following a feeding. Our observation of elevated CHO and RER in

348 the setting of greater hyperglycemia, suggesting greater metabolic flexibility in people
349 with higher body fat percentages, would fit the concept of metabolic flexibility precisely.
350 That is, they are adjusting substrate oxidation based on availability of glucose in the
351 blood for longer periods of time. Individuals with lower body fat percentages not
352 elevating CHO and RER during this period are likely experiencing the appropriate
353 catabolic (storage) response to feeding by taking up and storing glucose as glycogen
354 (liver and skeletal muscle) or fat (adipose tissue). As a result, they have no further need
355 to increase CHO to maintain blood glucose homeostasis.

356 This raises the question if our sample of young adults across the BMI spectrum
357 but with no overt metabolic pathology are demonstrating a resilient metabolically flexible
358 phenotype.[36] Metabolic flexibility is diminished following hypercaloric, high-fat feeding
359 interventions and improved following significant weight loss, indicating it is a plastic
360 phenotype.[37, 38] No study has successfully tracked the disappearance of metabolic
361 flexibility, but we suspect that long-term elevated levels of adiposity, such as that seen
362 in chronic obesity, eventually lead to the loss of postprandial metabolic flexibility. In our
363 sample, higher body fat percentage is inversely associated with insulin sensitivity ($r = -$
364 0.616; $p=0.001$) while being positively associated with fasting values for serum insulin
365 ($r=0.427$; $p=0.037$), plasma leptin ($r=0.817$; $p<0.001$), and adipose tissue insulin
366 resistance ($r=0.443$; $p=0.030$). These relationships may be indicative or worsening
367 metabolic health with higher body fat percentage, but the ability to elevate CHO
368 oxidation during the OGTT (e.g. metabolic flexibility) demonstrates a resilient,
369 metabolically flexibility phenotype in these individuals. The eventual loss of metabolic
370 flexibility would therefore play a significant role in the progression of glucose dysfunction

371 towards overt metabolic disease. That process and the mechanisms involved are still
372 poorly understood and require both experimental and observational longitudinal studies.

373 Our approach to analyzing metabolic flexibility during an OGTT as a continuous
374 variable across time is a novel aspect of our approach. Most studies assessing
375 adiposity and metabolic flexibility categorize subjects based on BMI, a surrogate for
376 adiposity, and define metabolic flexibility as the change in RER or CHO from fasting to a
377 defined timepoint (e.g. 60 min in an OGTT) or the peak change (max – min) in the
378 measure.[3] That approach largely demonstrates that individuals with overweight and
379 obesity have reduced (blunted) metabolic flexibility. Our data, when analyzed in that
380 way, produces similar results (**Supplementary Figure 2**). However, these indirect
381 associations do not clarify the relationship between adiposity and metabolic flexibility
382 and oversimplify the temporal postprandial metabolic response into a single timepoint,
383 leading to inconclusive results. Defining postprandial metabolic flexibility based on a
384 single timepoint during a dynamic and prolonged response, such as feeding, is likely a
385 carryover from the euglycemic clamp method, which applies indirect calorimetry to the
386 steady-state phase of insulin infusion. Because postprandial metabolism is dynamic, a
387 feeding challenge such as an OGTT has no equivalent to the steady-state period.[39, 40]
388 Using a continuous approach has allowed us to demonstrate an unexpected, but
389 explainable, relationship in which higher body fat percentage is associated with
390 postprandial metabolic flexibility in young adults without overt metabolic pathology.

391 In conclusion, we demonstrate that higher body fat percentage is associated
392 with greater postprandial metabolic flexibility (RER and CHO) during an oral glucose
393 tolerance test in young adults without overt metabolic pathology. We believe this finding

394 is indicative of a preserved metabolically flexible phenotype where elevated
395 postprandial blood glucose is resulting in elevated CHO with higher body fat
396 percentage. Our approach of analyzing data in a continuous manner using linear mixed
397 models, accounting for covariates known to modify the postprandial substrate oxidation
398 response, adds clarity to the previously poorly defined relationship between adiposity
399 and postprandial metabolic flexibility. While we cannot account for some functional (e.g.
400 adipocyte size, lipogenesis, and molecular) or anatomical (e.g. distribution) aspects of
401 adiposity, we directly demonstrate what has long been ambiguous in published reports.
402 These findings raise several new questions about adiposity and the continuum of
403 metabolic health.

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