Relatively low endogenous fatty acid mobilization and uptake helps preserve insulin sensitivity in obese women

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Abstract

Background—Although obesity is commonly linked with metabolic disease risk, some obese adults do not develop metabolic abnormalities, such as insulin resistance.

Objectives—The primary aim of this study was to determine whether alterations in fatty acid mobilization and uptake underlie differences in insulin sensitivity (S_i) among a seemingly homogeneous cohort of obese women.

Methods—Insulin sensitivity (FSIVGTT), basal fatty acid rate of disappearance from plasma (Rd), resting whole-body fat oxidation, intramyocellular triacylglycerol (IMTG) concentration, and markers of skeletal muscle inflammation were measured in 21 obese women. Participants were divided into tertiles based on their S_i. The subset of participants with the lowest S_i (LOW-S_i; S_i ≤2.1 (mU/L)⁻¹·min⁻¹; n=7) was compared with the subset of participants with the highest S_i, who exhibited relatively normal insulin sensitivity (NORM-S_i; S_i ≥3.4 (mU/L)⁻¹·min⁻¹; n=8).

Results—Despite nearly identical physical characteristics in LOW-S_i vs. NORM-S_i (BMI: 34±2 vs. 34±2 kg/m²; %body fat: 48±1% vs. 47±1%; waist circumference: 104±2 vs. 104±2 cm; VO₂max: 2.2±0.2 vs. 2.3±0.1 L/min), fatty acid Rd was nearly 30% lower in NORM (P=0.02). Importantly, the greater rate of fatty acid uptake in LOW-S_i vs. NORMS_i did not translate to higher rate of fat oxidation (3.5±0.2 vs. 3.7±0.2 μmol/kg/min) or to a measureable difference in IMTG content, (68.3±12.7 vs. 63.7±6.7 μmol/g dry weight). In conjunction with the lower fatty acid Rd in NORM-S_i vs. LOW-S_i, activation of inflammatory pathways known to impair insulin action in skeletal muscle was also lower (i.e. lower phosphorylated JNK, higher IκBα abundance). In contrast, LOW-S_i and NORM-S_i exhibited no differences in plasma markers of inflammation (i.e. TNFα, IL-6, MCP-1).

Conclusion—These findings suggest that obese women who maintain a relatively low rate of endogenous fatty acid uptake may be somewhat “protected” against the development of insulin resistance potentially by less activation of inflammatory pathways within skeletal muscle.
INTRODUCTION

Obesity is often associated with insulin resistance, which is a major contributor to the development of several chronic diseases, including type 2 diabetes. Despite the common link between obesity and disease risk, there are still many obese adults who appear to be somewhat “protected” against the development of many chronic health issues. In fact, it has been reported that over 30% of obese adults in the United States were classified as “metabolically healthy.” However, it is still unclear why some obese adults are more prone to develop metabolic complications, like insulin resistance, while others are not, even when they are very similar in terms of the magnitude and distribution of their adiposity.

Excessive systemic fatty acid availability and uptake has been identified as a key factor underlying insulin resistance in obesity. Indeed, abdominally obese individuals exhibit elevated systemic fatty acid availability compared with their lean counterparts, and lipid infusions in lean, healthy humans (used to elevate systemic fatty acid availability) readily impair insulin sensitivity. Conversely, acute reduction in systemic fatty acid availability (via lipolytic inhibition) can improve insulin sensitivity in obesity. Evidence suggests that the deleterious effects of elevated fatty acid availability and uptake are mediated in part via skeletal muscle accumulation of lipid intermediates, including diacylglycerol, ceramides, and fatty acyl-CoA. These intermediates can increase activation of pro-inflammatory pathways (e.g., c-jun N-terminal kinase (JNK), and inhibitor of κB/nuclear factor-κB) known to disrupt insulin signaling. Thus, elevated systemic fatty acid availability and subsequent skeletal muscle inflammatory pathway activation are believed to underlie the development of insulin resistance in obesity.

Although lipolytic rate and fatty acid availability are typically elevated in obesity, there is still considerable variability in fatty acid mobilization among the obese adults. It remains unclear whether the magnitude of endogenous fatty acid flux contributes to differences in the severity of insulin resistance among obese individuals. The primary aim of this study was to determine whether the magnitude of fatty acid uptake and markers of inflammatory pathway activation in skeletal muscle were related with the degree of insulin resistance in a cohort of obese sedentary women. We hypothesized that obese women with the lowest rates of fatty acid flux would exhibit relatively low pro-inflammatory pathway activation in skeletal muscle, and would be the least insulin resistant among the cohort.

METHODS

Participants

Twenty-one obese women (body mass index (BMI): 30–40 kg·m⁻²; waist circumference >100 cm) participated in this study. All women were premenopausal and considered to be in good health after a medical examination. The medical evaluation included a history
questionnaire, physical examination, 12-lead electrocardiogram, and standard blood and urine tests. None of the participants were taking regular medications except for some who were taking contraceptive medication. All participants were non-smokers, weight stable (±2 kg) for 6 months, and had not participated in regular exercise for at least 6 months before participating in the study. Participants with coronary heart disease, type 2 diabetes, hypertension, or clinically significant hypertriacylglycerolemia (plasma triacylglycerol > 150mg·dl$^{-1}$) were excluded. Some of these subjects participated in a previous study from our laboratory$^{17}$. All participants were fully informed of the possible risks associated with the study and signed an informed consent document, which was approved by the University of Michigan Institutional Review Board, in accordance with the Declaration of Helsinki.

Preliminary testing

Body composition was determined using dual x-ray absorptiometry (Lunar DPX DEXA Scanner, Madison, WI, USA). Aerobic fitness was analyzed by measuring peak oxygen uptake (VO$_2$ peak) during cycle ergometer exercise. The protocol consisted of a 4 minute warm-up, followed by a progressive increase in work rate every minute until volitional fatigue.

Experimental procedures

Participants were admitted to the Michigan Clinical Research Unit (MCRU) at the University of Michigan hospital at 1800h and stayed overnight. A standardized meal was provided at 2000h and the participants remained fasted until completion of the trial the next day.

At 0700h the next morning a skeletal muscle biopsy was obtained from the vastus lateralis. The muscle sample was separated from any adipose and connective tissue, rinsed with saline, blotted dry, frozen in liquid nitrogen, and stored at −80°C until analysis. Intravenous catheters were placed in a hand vein for blood sampling and in a forearm vein of the opposite arm for infusion of the stable isotope tracer, [13C]-palmitate (Cambridge Isotope Laboratories, Andover, MA, USA). A blood sample was obtained before the start of the tracer infusion to assess background [13C]-palmitate enrichment and to measure baseline concentrations of plasma pro-inflammatory markers (i.e., TNF-a, IL-6, and MCP-1). At 0800h, a constant infusion of [13C] palmitate (0.04 μmol kg$^{-1}$ min$^{-1}$) bound to human albumin (Baxter, Deerfield, IL, USA) was initiated. Four arterialized blood samples were collected in 5 min intervals from a heated hand vein at minutes 45, 50, 55, and 60 for determination of the rate of fatty acid disappearance from plasma (Rd), to assess fatty acid uptake. Resting whole-body fat oxidation was calculated from the rates of oxygen consumption (VO$_2$) and carbon dioxide production (VCO$_2$) using a metabolic cart (Delta Trac, Sensor Medics Yorba Linda, CA, USA). After all of the fatty acid metabolism measurements, a frequently sampled intravenous glucose tolerance test (FSIVGTT) was conducted to assess insulin sensitivity ($S_i$) using the minimal model technique, as previously described$^{18}$. The trial was performed during the first 2 weeks of the participants’ menstrual cycle.
Analytical procedures

Plasma fatty acid kinetics—The tracer-to-tracee ratio (TTR) for plasma palmitate was determined by gas chromatography-mass spectrometry (GC-MS) with an MSD 5973 system (Agilent Technologies; Wilmington, DE, USA) with capillary column as previously described\(^\text{19,20}\). Plasma palmitate concentration was measured by the internal standard method using GC/flame ionization detection (GC/FID)\(^\text{21}\).

Plasma glucose, fatty acid and insulin concentrations—Plasma glucose (Thermo Scientific, Waltham MA, USA) and fatty acid (Wako Chemicals USA, Richmond, VA, USA) concentrations were measured using a commercially available colorimetric assay kits. Plasma insulin concentration was measured by radioimmunoassay (Linco Research Inc., St Louis, MO, USA).

Intramyocellular triacylglycerol concentration—Skeletal muscle triacylglycerol concentration was determined with saponification techniques as previously described\(^\text{20,22}\). Briefly, triacylglycerols were extracted from the dried muscle sample using a 2:1 chloroform:methanol solution and saponified in 4% ethanolic KOH. Free glycerol concentration was then determined fluorometrically.

Tissue lysate preparation—Frozen muscle samples were weighed and transferred into pre-chilled microfuge tubes containing 1 ml ice cold lysis buffer and a steel ball bearing. Tissue samples were homogenized in the microfuge tubes for 5 minutes using a Qiagen TissueLyser II (Qiagen, Hilden, Germany). The lysis buffer contained T-PER Tissue Protein Extraction Reagent (#78510, Fisher Scientific, USA), 1mM EDTA, 1mM EGTA, 2.5 mM sodium pyrophosphate, 1mM sodium orthovanadate, 1mM β-glycerophosphate, 1 μg/ml leupeptin, and 1mM phenylmethylsulfonyl fluoride. In order to remove insoluble material, the homogenates were transferred to new microfuge tubes and rotated for one hour at 4°C and then centrifuged at 15,000 g for 15 min at 4°C. Protein concentration was measured using the bicinchoninic acid method (Thermo Scientific, Rockford, IL, USA).

Multiplex analysis—Plasma cytokines and skeletal muscle proteins associated with inflammation were quantified using a commercially available Multiplex bead assay per manufacturer recommendations (Luminex L200, Luminex, Austin, TX, USA). Plasma was assayed for IL-6, TNF-α, and MCP-1 as (#HCVD3-67CK, Milliplex MAP Kit, Millipore, Billerica, MA, USA). Prepared tissue lysates were analyzed for total protein abundance of JNK and IkBα and separately analyzed for the total phosphorylation of JNK\(^\text{Thr185/Tyr185}\) (#48-602, Milliplex MAP Kit, Millipore, Billerica, MA, USA).

Calculations

Fat oxidation—Whole body fat/triacylglycerol oxidation (g/min) was calculated from VO\(_2\) and VCO\(_2\) measurements using the equations of Frayn\(^\text{23}\). Whole body fatty acid oxidation was calculated by dividing triacylglycerol oxidation by an estimated molecular weight of triacylglycerol (860 g/mol) and multiplying by 3.
Fatty acid uptake—Palmitate rate of disappearance (Rd) from plasma was calculated using the Steele equation for steady-state conditions. Fatty acid Rd was calculated by dividing palmitate Rd by the ratio of plasma palmitate to total plasma fatty acid concentration.

Insulin sensitivity index (S_i)—The insulin sensitivity index (S_i) was calculated from least squares fitting of the insulin and glucose concentration curves from the IVGTT using the Minimal Model Millenium (version 6.02; MiniMod Inc.) computer analysis software. Although not the gold standard, S_i has been found to match well with insulin sensitivity measured by the hyperinsulinemic-euglycaemic clamp technique.

Fatty acid area under the curve during the IVGTT—Area under the curve (AUC) for plasma fatty acid concentration during time 0–180 min of the IVGTT was calculated using the trapezoidal rule.

Statistics

Unpaired Student’s t-tests were used to test for significant between group (NORM-S_i vs. LOW-S_i) differences in outcome variables. Simple linear regression was used to examine the relationship between S_i, fatty acid uptake, IκBα, and JNK in all participants (n=21). Statistical significance was defined as P < 0.05. All results are presented as mean ± standard error (SE). All analysis was performed on SigmaPlot version 11.0 computer software.

RESULTS

Insulin sensitivity index and cohort stratification

As designed, the participant pool was largely homogeneous in terms of BMI, adiposity, waist circumference, and cardiorespiratory fitness (Table 1); however, S_i varied widely among the 21 participants (Figure 1), ranging from 4.8 to 0.8 (mU/L)^{-1}.min^{-1}. As noted in Figure 1A, participants with S_i in the lowest one-third of the overall participant pool (≤2.1 (mU/L)^{-1}.min^{-1}) were grouped into the “low” insulin sensitive cohort (LOW-S_i; n=7), and those in the highest one-third (≥3.4 (mU/L)^{-1}.min^{-1}) were grouped into the “normal” insulin sensitivity cohort (NORM-S_i; n=8). The term “normal” was used to define the S_i of the most insulin sensitive participants because these values were very similar to those previously reported by our laboratory and others for lean, healthy adults.

As designed, the difference in S_i between NORM- S_i and LOW- S_i was highly significant (Figure 1B; P<0.000001); but importantly, these groups were very well matched for BMI, adiposity, waist circumference, and cardiorespiratory fitness (Table 1). In addition, fasting plasma glucose and insulin concentrations were similar in NORM-S_i and LOW-S_i (Table 1; p=0.47, p=0.28, respectively). In order to compare groups with distinct differences in insulin sensitivity, primary comparisons did not include participants with S_i values between 2.1 and 3.4 (mU/L)^{-1}.min^{-1} (grey bars in Figure 1). The participants with intermediate S_i were included in correlation analyses, which incorporated the entire participant pool. The racial profile within our groups were as follows: NORM-S_i - 2 African American and 6 Caucasian women; LOW-S_i –1 African American, 1 Asian, 1 Hispanic/Latino and 4 Caucasian women; Intermediate-S_i – 2 African American and 4 Caucasian women.
Fat metabolism

In conjunction with higher insulin sensitivity, fatty acid Rd was nearly 30% lower in NORM-Si vs. LOW-Si (Figure 2; P<0.02). Because we measured fatty acid kinetics under steady-state conditions, fatty acid Rd was equivalent to the rate of fatty acid appearance into the systemic circulation. Therefore, not only is fatty acid uptake lower in NORM-Si vs. LOW-Si, but fatty acid mobilization from adipose tissue was lower as well. Despite the lower rates of fatty acid mobilization and uptake in NORM-Si vs. LOW-Si, resting whole-body fatty acid oxidation was not different between groups (3.7 ± 0.2 vs. 3.5 ± 0.2 μmol/kg/min, respectively). Interestingly, fatty acid uptake was reasonably well matched to the rate of fatty acid oxidation in NORM-Si (3.8 ± 0.5 vs. 3.7 ± 0.2 μmol/kg/min, respectively), but in LOW-Si the rate of fatty acid uptake exceeded fatty acid oxidation by nearly 50% (5.2 ± 0.4 vs. 3.5 ± 0.2 μmol/kg/min, respectively). However, this disparity between fatty acid uptake and fat oxidation in LOW-Si did not translate to a measureable difference in IMTG content, which was similar between groups (63.7 ± 6.7 vs. 68.3 ± 12.7 μmol/g dry weight for NORM-Si and LOW-Si, respectively). In addition to the lower rate of fatty acid uptake in the overnight fasted state in NORM-Si compared with LOW-Si, plasma fatty acid concentration during the IVGTT was also significantly lower in NORM-Si vs. LOW-Si in response to insulin during the IVGTT (AUC: 29±10 vs. 47±15 mM•min, respectively, P=0.02; Figure 3).

Markers of inflammation

Within skeletal muscle, JNK phosphorylation (p-JNK) was significantly lower in NORM-Si compared with LOW-Si (Figure 4A), suggestive of attenuated inflammatory pathway activation in muscle from NORM-Si participants. This may have been due in part to a lower total JNK protein abundance in NORM-Si vs. LOW-Si (101±10 vs. 149±20 arbitrary units (AU), respectively; p ≤0.05). Skeletal muscle protein abundance of IκB-α tended to be greater in NORM-Si compared with LOW-Si (Figure 4B), but this difference between groups did not quite reach statistical significance (p=0.067). Because IκB-α suppresses activation of the IKK-NFκB inflammatory pathway, the trend for greater abundance of IκB-α in muscle from NORM-Si vs. LOW-Si is also indicative of reduced inflammation in NORM-Si. In contrast to the differences in markers of inflammation in skeletal muscle, there were no significant differences in fasting plasma concentrations of IL-6 (15.0 ± 8.4 vs. 23.8 ± 10.0 pg/ml), TNF-α (4.3 ± 1.0 vs. 7.9 ± 2.2 pg/ml), or MCP-1 (128.5 ± 12.6 vs. 114.5 ± 32.7 pg/ml) between NORM-Si and LOW-Si, respectively.

Relationships between insulin sensitivity, fatty acid uptake, and inflammatory markers

Among the entire participant population (n=21), fatty acid uptake was inversely correlated with Si (Figure 5A; R²=0.21; p = 0.04), further supporting the notion that those with relatively low fatty acid uptake were the most insulin sensitive. Similarly, skeletal muscle IκB-α content was positively correlated with Si (Figure 5B; R²=0.22; p=0.035) and skeletal muscle p-JNK tended to be inversely correlated with Si (Figure 5C; R² = 0.18; p = 0.06), both suggesting that lower inflammatory pathway activation in skeletal muscle is related to insulin sensitivity in obesity. We found no correlations between IL-6, MCP-1, or TNF-α in relation to fatty acid uptake (IL-6: R²=0.052, p=0.25; MCP-1: R²=0.0004, p=.94; TNF-α:
DISCUSSION

While many obese adults have serious cardio-metabolic complications and/or are at high risk for developing chronic diseases, such as type 2 diabetes, there are some obese adults who appear to be in relatively good health. We found that just over one-third of our obese cohort exhibited “normal” insulin sensitivity, which matched very well with epidemiological evidence using data collected from the National Health and Nutrition Examination Survey (NHANES). However, it is not clear why some obese adults are prone to develop insulin resistance (as well as other cardio-metabolic complications), while others appear to remain somewhat “protected”. In our participants, body mass, BMI, waist circumference, fat mass, fat free mass, cardiorespiratory fitness, resting fat oxidation, and skeletal muscle triacylglycerol content could not explain the disparity in insulin resistance. Instead, our main findings suggest that a relatively low basal rate of fatty acid uptake may be a key factor helping to preserve insulin sensitivity in some obese adults.

An overabundance of fatty acids has long been known to induce insulin resistance. Excessive fatty acid uptake into insulin responsive tissues like skeletal muscle is thought to induce insulin resistance in part via the intracellular accumulation of lipid intermediates, such as diacylglycerol, ceramides, and fatty acyl-CoA. Theoretically, high rates of fatty acid oxidation may help compensate for elevated fatty acid uptake, thereby attenuating the accumulation of lipid intermediates, which may help protect against the development of insulin resistance. However, the impact that variation in resting fatty acid oxidation may have on the development of insulin resistance is questioned. In our study, rates of fatty acid oxidation were identical in NORM-S and LOW-S, suggesting differences in oxidative disposal of fatty acids was not likely contributing to the differences in insulin sensitivity observed between these groups. In contrast, a ~40% greater rate of fatty acid uptake in the LOW-S vs. NORM-S contributed to a large mismatch between the rates of fatty acid uptake and oxidation in this group. Although we did not measure the muscle accumulation of diacylglycerol, ceramide, or fatty acyl-CoA in this study due to limitations in the amount of tissue available, it is plausible that a disparity between fatty acid uptake and oxidation of this magnitude may have led to an accumulation of these lipid intermediates. In turn, the accumulation of these lipids within the muscle cell can increase the activation of inflammatory pathways, which may contribute to the impairment in insulin action.

It has become evident that increased inflammatory pathway activation in skeletal muscle is an important link between altered fatty acid metabolism and impaired insulin signaling. The high rates of fatty acid mobilization and uptake commonly found in obesity is often accompanied by an elevated activation of JNK and/or IKK in skeletal muscle. Importantly, pharmacologically induced suppression of fatty acid availability in humans was found to suppress the activation of inflammatory pathways in skeletal muscle, and improve insulin action. Furthermore, previous work from our laboratory demonstrated that the improvement in insulin sensitivity found after weight loss was largely attributed to the weight loss-induced reduction in fatty acid availability, with an
accompanying decline in the activation of the inflammatory JNK and IKK-NFκB pathways\textsuperscript{17}. Our present finding that the relatively low rate of fatty acid uptake in NORM-S\textsubscript{i} was accompanied by lower p-JNK and a higher IκB\textalpha abundance compared with LOW-S\textsubscript{i}, highlights the importance of the magnitude of fatty acid uptake as a key mediator of inflammation and insulin sensitivity within skeletal muscle.

When examining health disparities among obese adults, there is considerable emphasis on the role that differences in anatomical distribution of body fat (i.e., subcutaneous vs. visceral) may play in determining metabolic health in obesity. Abdominal obesity is most commonly linked with insulin resistance\textsuperscript{40,41}, as well as other cardiovascular disease risk factors\textsuperscript{42,43}. More specifically, accumulation of fat in the visceral region is typically identified as being an especially potent contributor to the development of metabolic disorders\textsuperscript{44–46}. Our NORM-S\textsubscript{i} and LOW-S\textsubscript{i} groups were tightly matched for waist circumference, which provides a reasonably accurate surrogate measure for visceral adiposity\textsuperscript{47}. Nevertheless, because we did not definitively assess visceral fat mass, we cannot rule out the possibility that potential differences in visceral adiposity may help explain some of the observed difference in insulin sensitivity among our participants. Many reports suggest that visceral adiposity contributes to insulin resistance as a consequence of the relatively high lipolytic rate measured in visceral compared with subcutaneous adipocytes\textsuperscript{48,49}. However, in vivo measurements indicate that about 85% of systemic fatty acids are derived from subcutaneous adipose tissue, whereas only about 15% come from visceral adipose tissue\textsuperscript{50}. Therefore, differences in the regulation of fatty acid release from subcutaneous (rather than visceral) adipose tissue are likely responsible for the observed disparity in systemic fatty acid mobilization between NORM-S\textsubscript{i} and LOW-S\textsubscript{i}.

The rate of fatty acid mobilization from subcutaneous adipose tissue has been reported to vary widely among obese adults\textsuperscript{15,16}. The rate of fatty acid release from adipose tissue is determined in large part by the rate of lipolysis, which liberates fatty acids from triacylglycerol. Therefore, a relatively low lipolytic rate within adipose tissue would suppress the mobilization of fatty acids into the systemic circulation, which may lower the risk for developing insulin resistance. Along these lines, it has recently been reported that specific inhibition of hormone sensitive lipase in mice decreased fatty acid mobilization and lead to an increase in insulin sensitivity\textsuperscript{16}. These same authors also found lipolytic rate to be positively correlated with insulin resistance in humans, independent of BMI\textsuperscript{16}. Importantly, the rate of triacylglycerol synthesis (i.e., fatty acid esterification) can also modulate the rate fatty acid release, and it has been reported that obese individuals with a relatively high capacity for triacylglycerol synthesis in subcutaneous adipose tissue were largely protected from insulin resistance\textsuperscript{15}. Future studies designed to examine the abundance and activity of key factors that regulate fatty acid release and triacylglycerol synthesis in adipose tissue will help identify potential mechanisms mediating the differences in fatty acid mobilization and uptake in obesity.

Insulin potently suppresses lipolysis\textsuperscript{51}, therefore individual variability in the magnitude of the insulin-mediated suppression of fatty acid mobilization may have also contributed to the variability in insulin resistance among our participants. Our finding that plasma fatty acid concentration during the IVGTT was lower in NORM-S\textsubscript{i} vs LOW-S\textsubscript{i} suggests that LOW-S\textsubscript{i}
may have also been more resistant to the anti-lipolytic effects of insulin than NORM-Si. This is in accord with recent findings demonstrating that the magnitude of the insulin-induced suppression in fatty acid mobilization was significantly correlated with insulin-mediated glucose uptake\(^5^2\). Interestingly, despite also demonstrating a significant relationship between post-absorptive fatty acid availability and insulin resistance (similar to our present findings), the authors challenged the notion that fatty acid mobilization in the post-absorptive state was an important contributor to the development of insulin resistance\(^5^2\). We agree that if fatty acid availability remains relatively high despite insulin exposure (e.g., after a meal, during an IVGTT, or hyperinsulinemic clamp) – this certainly could contribute to the development of insulin resistance. However, even though the anti-lipolytic response to insulin appeared to be blunted in LOW-Si vs. NORM Si, plasma fatty acid concentration during the IVGTT was still suppressed to very low levels in LOW-Si (i.e., 60–80% below overnight fasted concentrations (Figure 3)). If the accumulation of lipid intermediates within the muscle cell does indeed play an important role in the development of insulin resistance\(^1^0, ^1^1\), these intracellular lipids likely accumulate in large part in the post-absorptive state, when fatty acid availability is highest, and muscle fatty acid oxidation does not increase sufficiently to accommodate the high rate of fatty acid uptake. Therefore, we contend that the exposure to high availability of fatty acids that is often found in the post-absorptive state of many obese adults can have a potent impact on insulin resistance.

Interestingly, we did not find any differences in plasma cytokines known to be involved with systemic inflammation between the NORM-Si and LOW-Si groups. The chronic low-grade inflammation observed in obesity is partly attributed to elevated systemically circulating inflammatory cytokines such as TNF-\(\alpha\) and Interleukin 6 (IL-6)\(^5^3, ^5^4\). We can possibly attribute these results to our relatively small sample size and the inherent variability typically observed in these circulating cytokines. Alternatively, it may be indicative that the inflammatory response is primarily mediated within the peripheral tissues as opposed to the systemically circulating cytokines. However, it is possible that other cytokines and/or adipokines not measured in this study (i.e. adiponectin, IL-1\(\beta\), etc.) may be different.

In conclusion, our findings indicate that variability in systemic fatty acid uptake is a key mediator of the observed differences in insulin resistance among sedentary obese adults. More specifically, obese participants who had a relatively low rate of fatty acid uptake appear to be somewhat “protected” against the development of insulin resistance. This lower rate of fatty acid uptake was accompanied by a lower activation of markers for inflammatory pathways within the skeletal muscle, and consequently, higher insulin sensitivity when compared with our participants with relatively high rates of fatty acid uptake. Importantly, the observed variability in insulin resistance among our obese participants could not be attributed to differences in basal fat oxidation, common markers of systemic inflammation (e.g., IL-6, TNF-\(\alpha\), MCP-1), skeletal muscle triacylglycerol accumulation, or cardiorespiratory fitness. It is likely that differences in the regulation of fatty acid storage and release from adipose tissue mediates a large portion of this observed variability in fatty acid availability and uptake. Therefore, it is now very important to identify the factors underlying the relatively low fatty acid availability and uptake among obese individuals who are not insulin resistant (and/or factors underlying the high fatty acid availability among
those who are) in order to develop preventative, and/or therapeutic approaches in the treatment of insulin resistance and related diseases.

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Figure 1. Insulin sensitivity

(A) Insulin sensitivity index ($S_i$) measured the morning after an overnight fast in all subjects (n=21). Subjects were ordered from highest to lowest $S_i$ and stratified into tertiles to identify a low insulin sensitivity cohort (LOW-$S_i$: $S_i \leq 1.1$ (mU/L)⁻¹.min⁻¹; n=7) and a normal insulin sensitivity cohort (NORM-$S_i$: $S_i \geq 3.4$ (mU/L)⁻¹.min⁻¹) (B) mean $S_i$ in NORM-$S_i$ and LOW-$S_i$ cohorts. *$P < 0.000001$ vs. NORM-$S_i$.
Figure 2. Fatty acid uptake
Fatty acid rate of disappearance from plasma (Rd) in NORM-S₁ and LOW-S₁ cohorts, measured the morning after an overnight fast. *P < 0.05 vs. NORM- S₁
Figure 3. Insulin-induced suppression of plasma fatty acid concentration

Plasma fatty acid concentration during the 3h intravenous glucose tolerance test (IVGTT) in LOW-S_i (white square □) and NORM-S_i (black square ■). Values are mean ± SE. * Significant difference in Area under the curve (AUC) between the groups, P = 0.02.
Figure 4. Markers of inflammation
(A) Total protein abundance of phosphorylated c-Jun N-terminal kinase (p-JNK) (B) Total protein abundance of Inhibitor of NF-κB α (IκB-α). *P < 0.05 for NORM-Si vs. LOW-Si
Figure 5. Correlational analyses for insulin sensitivity with fatty acid uptake and inflammatory markers in skeletal muscle

(A) Correlation between insulin sensitivity index (Si) and fatty acid uptake. (B) Correlation between Si and IκB-α abundance. (C) Correlation between Si and p-JNK abundance. All correlational analyses were performed using the entire subject cohort (n=21) [LOW-Si (n=7; white square □), NORM-Si (n=8; black square ■) and participants that did not fall into either category (n=6; grey square □)].
Table 1
Subject Characteristics

|                        | All Subjects | NORM-S_i | LOW-S_i |
|-----------------------|--------------|----------|---------|
| Age (y)               | 30 ± 1       | 32 ± 2   | 29 ± 3  |
| Body Mass (kg)        | 93 ± 2       | 92 ± 2   | 91 ± 5  |
| BMI (kg/m²)           | 34 ± 1       | 34 ± 1   | 34 ± 2  |
| Waist circumference (cm) | 104 ± 1     | 104 ± 2  | 104 ± 2 |
| Fat Mass (kg)         | 44 ± 2       | 44 ± 1   | 44 ± 4  |
| Fat free mass (kg)    | 49 ± 1       | 49 ± 1   | 48 ± 2  |
| Body Fat (%)          | 48 ± 1       | 47 ± 1   | 48 ± 1  |
| VO₂peak (ml/kg/min)   | 24 ± 1       | 25 ± 1   | 23 ± 1  |
| Fasting glucose (mM)  | 4.8 ± 0.2    | 5.0 ± 0.2| 4.7 ± 0.4|
| Fasting insulin (μU/mL) | 14.2 ± 0.9  | 15.2 ± 1.2| 13.3 ± 1.2 |
| HOMA-IR               | 3.0 ± 0.2    | 3.4 ± 0.3| 2.7 ± 0.4|