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ORIGINAL ARTICLE

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Metabolic dysfunction in obesity is related to impaired suppression of fatty acid release from adipose tissue by insulin

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Abstract

Objective: The aims of this study were: 1) to assess relationships among insulin-mediated glucose uptake with standard clinical outcomes and deep-phenotyping measures (including fatty acid [FA] rate of appearance [FA Ra] into the systemic circulation); and 2) to examine the contribution of adipocyte size, fibrosis, and proteomic profile to FA Ra regulation.

Methods: A total of 66 adults with obesity (BMI = 34 [SD 3] kg/m^2) were assessed for insulin sensitivity (hyperinsulinemic-euglycemic clamp), and stable isotope dilution methods quantified glucose, FA, and glycerol kinetics *in vivo*. Abdominal subcutaneous adipose tissue (aSAT) and skeletal muscle biopsies were collected, and magnetic resonance imaging quantified liver and visceral fat content.

Results: Insulin-mediated FA Ra suppression associated with insulin-mediated glucose uptake ($r = 0.51$; $p < 0.01$) and negatively correlated with liver ($r = -0.36$; $p < 0.01$) and visceral fat ($r = -0.42$; $p < 0.01$). aSAT proteomics from subcohorts of participants with low FA Ra suppression ($n = 8$) versus high FA Ra suppression ($n = 8$) demonstrated greater extracellular matrix collagen protein in low versus high FA Ra suppression. Skeletal muscle lipidomics ($n = 18$) revealed inverse correlations of FA Ra suppression with acyl-chain length of acylcarnitine ($r = -0.42$; $p = 0.02$) and triacylglycerol ($r = -0.51$; $p < 0.01$), in addition to insulin-mediated glucose uptake (acylcarnitine: $r = -0.49$; $p < 0.01$, triacylglycerol: $r = -0.40$; $p < 0.01$).

Conclusions: Insulin's ability to suppress FA release from aSAT in obesity is related to enhanced insulin-mediated glucose uptake and metabolic health in peripheral tissues.

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See Commentary, pg. 1185.

INTRODUCTION

Impaired insulin-mediated glucose uptake (i.e., insulin resistance) underlies many obesity-related diseases [1]. Although most adults with obesity are resistant to the glucose-lowering effects of insulin, up to 30% of adults with obesity remain relatively insulin sensitive, with minimal metabolic health complications [2]. Why some adults with obesity are profoundly resistant to insulin-mediated glucose uptake, whereas others are relatively insulin sensitive, remains elusive. Findings from our lab [3, 4] and others [5, 6] have suggested that maintaining low fatty acid (FA) mobilization rates into the systemic circulation may "protect" some adults with obesity from developing insulin resistance. The majority of FA delivered into the systemic circulation is derived from subcutaneous adipose tissue (SAT) depots localized to the upper body, rather than gluteal or visceral depots [7]. The rate of FA mobilization from abdominal SAT (aSAT) into the systemic circulation is primarily determined by triacylglycerol (TAG) hydrolysis (lipolysis) and the rate at which FA liberated by lipolysis is reincorporated into TAG within adipocytes (reesterification). Although these processes are largely regulated by lipase and acyltransferase enzyme activity, other factors such as aSAT morphology (i.e., size, vascularization, fibrosis) and local and systemic inflammation may also impact FA release [8, 9].

The metabolic consequences of excessively high FA availability contribute to high FA uptake rates and resultant "ectopic" lipid accumulation in other tissues such as skeletal muscle and liver [10]. High rates of FA uptake into the liver can impair hepatic insulin sensitivity and lead to the development of chronic liver disorders (e.g., nonalcoholic fatty liver disease, steatohepatitis). Because skeletal muscle is the primary site of insulin-mediated glucose uptake [11], preservation of skeletal muscle insulin action is central for maintaining whole-body glucose control. Excessive FA uptake into skeletal muscle can lead to a local accumulation of lipid intermediates, including diacylglycerol (DAG) [12], ceramide [13], and long-chain acyl-coenzyme A (CoA) [14, 15], which have been causally linked with insulin resistance. Additionally, the conformation of lipid species such as acyl-chain length [16] and saturation [17] has been implicated in skeletal muscle insulin signaling. Given the important repercussions of high FA uptake and lipid accumulation in ectopic tissues, an enhanced ability to sequester FA in aSAT appears metabolically favorable. However, the relationships among aSAT FA mobilization and lipid accumulation in these tissues remain unresolved.

The primary aims of this study were as follows: 1) to assess relationships among insulin-mediated glucose uptake with standard clinical outcomes, as well as deep-phenotyping measures, including FA rate of appearance (FA Ra) into the systemic circulation; and 2) to examine the potential contribution of adipocyte size, extracellular matrix (ECM) collagen accumulation, and aSAT proteomic profile to FA Ra in adults with obesity and wide-ranging insulin sensitivity. We also examined relationships among FA Ra with lipid accumulation in skeletal muscle, liver, and intra-abdominal adipose tissues.

Study Importance

What is already known?

- Rates of fatty acid (FA) release from abdominal subcutaneous adipose tissue (aSAT) can vary considerably among individuals with obesity.
- Excessive rates of FA release from aSAT and resultant uptake into metabolically active tissues such as skeletal muscle and liver are key factors underlying the development of insulin resistance in obesity.

What does this study add?

- The suppression of FA rate of appearance in response to insulin (FA Ra suppression) associated with insulin-mediated glucose uptake and the ectopic accumulation of long-chain acylcarnitine and triacylglycerol in skeletal muscle.
- Factors associated with aSAT morphology, such as smaller adipocyte size and lower extracellular matrix fibrosis, may contribute to enhanced FA Ra suppression in response to insulin.

How might these results change the direction of research or the focus of clinical practice?

- Greater FA Ra suppression in response to insulin may be attributed to lower aSAT fibrosis and adipocyte size in adults with obesity, as well as greater metabolic health outcomes.

METHODS

Study population

A total of 66 men and women ($n = 20$, $n = 46$, respectively) with obesity (body mass index [BMI] = 30-40 kg/m^2) completed the study (Table 1). All participants were weight stable (±2 kg) and sedentary (no planned moderate-to-vigorous exercise for previous 6 months). After completion of the testing reported here, a subset of participants ($n = 36$) subsequently participated in an exercise training intervention addressing hypotheses unrelated to the present work [18, 19]. Participants were not taking medications known to affect glucose or lipid metabolism and they did not have a history of heart disease or active smoking. All women were premenopausal, eumenorrheic, and not pregnant or lactating. All participants provided written informed consent before participation. The study protocol was approved by the University of Michigan Institutional Review Board, carried out in accordance with the principles of the Declaration of Helsinki, and registered at ClinicalTrials.gov (NCT02717832, NCT02706093).

TABLE 1 Participant characteristics from the entire cohort and subcohort analysis

	All participants	Subcohort analysis		
	$n = 66$, F = 46, M = 20	HS ($n = 8$), F = 6, M = 2	LS ($n = 8$), F = 6, M = 2	*p* value
Age (y)	31 ± 7	31 ± 6	32 ± 5	0.85
Body mass (kg)	96.9 ± 12.2	94.7 ± 9.6	94.9 ± 8.7	0.96
Fat mass (kg)	42.1 ± 6.7	40.7 ± 4.7	40.8 ± 5.8	0.99
Fat-free mass (kg)	54.7 ± 9.5	53.9 ± 9.7	54.2 ± 9.1	0.96
Body fat (%)	43.5 ± 5.4	43.3 ± 5.7	43.1 ± 6.1	0.95
BMI (kg/m^2)	34.0 ± 3.0	34.0 ± 2.6	33.8 ± 2.6	0.87
Glucose (mmol/L)	4.9 ± 0.5	4.6 ± 0.4	4.8 ± 0.6	0.40
Insulin (μU/mL)	16.2 ± 9.7	7.0 ± 2.2	19.2 ± 10*	<0.01
HbA_{1c} (%)	5.3 ± 0.4	5.1 ± 0.2	5.5 ± 0.6	0.13
HOMA-IR	3.6 ± 2.3	1.4 ± 0.5	4.2 ± 2.5*	0.010
NEFA (μmol/L)	432 ± 169	378 ± 99	403 ± 164	0.72
Triacylglycerol (mmol/L)	0.98 ± 0.6	0.6 ± 0.2	1.1 ± 0.4*	0.015
HDL cholesterol (mmol/L)	1.02 ± 0.3	1.17 ± 0.4	0.95 ± 0.2	0.17

Note: Values are expressed as mean ± SD. Plasma concentrations were evaluated after an overnight fast.
Abbreviations: F, female; FA, fatty acid; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; HS, high FA Ra suppression; LS, low FA Ra suppression; M, male; NEFA, nonesterified fatty acid; Ra, rate of appearance.
*$p < 0.05$ versus HS subcohort.

Experimental protocol

Participants consumed a standardized dinner and snack the day before the study trial and arrived at the Michigan Clinical Research Unit (MCRU; Ann Arbor, Michigan) at 0700 h the following morning (Supporting Information Figure S1 presents study schematic and details). After 30 minutes of rest, resting metabolic rate and substrate (fat and carbohydrate) oxidation were measured via indirect calorimetry (Vmax; CareFusion, San Diego, California). Resting metabolic rate (kilocalories per day) was calculated from the Weir equation [20], and substrate oxidation (grams per minute) was calculated according to Frayn [21]. At ~0800 h, intravenous catheters were inserted into the hand/forearm vein for continuous blood sampling and continuous isotope, insulin, and glucose infusion. At ~0900 h, baseline blood samples were collected for background isotope enrichment, followed by primed continuous infusions of [6,6 $^2H_2$]glucose (35 μmol/kg priming dose; 0.41 μmol/kg/min continuous infusion) and [$^2H_5$]glycerol (1.5 μmol/kg priming dose; 0.1 μmol/kg/min continuous infusion). At ~0915 h, skeletal muscle biopsies were collected from the vastus lateralis, and aSAT biopsies were collected by aspiration lateral to the umbilicus. At ~1000 h, a continuous [1-^{13}C]palmitate infusion (0.04 μmol/kg/min) began to determine FA kinetics. Three arterialized samples (heated-hand technique) were collected at 1050 h, 1055 h, and 1100 h to determine fasting substrate kinetics. At ~1100 h, a 2-hour hyperinsulinemic-euglycemic clamp procedure began to determine insulin-mediated glucose uptake (40 mU/m^2/min). Continuous blood glucose samples were obtained every 5 minutes (StatStrip; Nova Biomedical, Waltham, Massachusetts), and dextrose (20% dextrose, enriched with [6,6 $^2H_2$]glucose) infusion rates were modified to maintain baseline glucose concentration at ~5 mmol/L. Five blood samples were collected during the last 20 minutes of the clamp to determine insulin-mediated substrate kinetics.

Body composition, liver fat, and visceral fat area

Body composition was assessed by dual-energy x-ray absorptiometry (Lunar DPX; GE Healthcare, Madison, Wisconsin) at the MCRU. Visceral fat area and liver fat percentage were measured by magnetic resonance imaging (MRI; Ingenia 3T; Philips, Amsterdam, Netherlands) at the University of Michigan, Department of Radiology. MRI images were captured by chemical-shift-encoded MRI proton density fat fraction [22], and image acquisition was performed as described previously [18]. Visceral fat area was measured from 3 axial sections (5 mm thickness) in the L2-3 interspace, and liver fat percentage was measured from 3 liver axial sections (5 mm thickness). Both liver and visceral fat area was quantified by a trained investigator blinded to participant identification.

Participant stratification into high versus low FA Ra suppression subcohorts

For paired analyses, a cohort of participants was stratified into high FA Ra suppression (HS) or low FA Ra suppression (LS) subcohorts based on percent change in FA Ra from baseline to hyperinsulinemia during the clamp (HS = 85% ± 2%; LS = 63% ± 6% FA Ra suppression by insulin; $n = 8$ per subcohort). Importantly, participants selected for subcohort stratification were strictly matched for sex and fat mass to

avoid confounding effects of these parameters on metabolic health (Table 1).

Plasma glucose, glycerol, and FA kinetics

Tracer to tracee ratios quantified glucose, palmitate, and glycerol kinetics by gas chromatography-mass spectrometry (GC-MS) (described in online Supporting Information Methods). Kinetics were calculated respective to tracer to tracee ratios from each substrate by the Steele equation for steady-state conditions [23]. FA Ra was quantified by dividing palmitate Ra by the ratio of total palmitate (C16:0) to FA within a standard, as described previously [3, 4].

Adipocyte size and fibrosis

Adipocytes were stained by Harris's hematoxylin (Sigma-Aldrich, St. Louis, Missouri) and eosin (Sigma-Aldrich), as described previously by our lab [19]. Adipocyte size was quantified using Image J (National Institutes of Health, Bethesda, Maryland), as described previously [24]. Large and small adipocyte distributions were determined by the lowest quartile (Q1; 1000-3196 μm^2) and highest quartile (Q4; >6784 μm^2) from the entire cohort. For fibrosis measurements, picrosirius red-stained samples were normalized to tissue surface area from 10 random fields at 10× magnification, as described previously [25].

Plasma metabolite, cytokine, and hormone concentration

Plasma glucose, nonesterified FA, TAG, high-density lipoprotein cholesterol, and total cholesterol were measured using commercially available colorimetric assays. Plasma interleukin 6 (IL-6), C-reactive protein, leptin, total adiponectin, and high-molecular-weight adiponectin were measured by enzyme-linked immunosorbent assay (ELISA). Insulin was measured by radioimmunoassay (RIA). Supporting Information Table S1 includes vendor details.

aSAT proteomics

Liquid chromatography–tandem mass spectrometry proteomic analysis and quantification were completed by the Proteomics Resource Facility at the University of Michigan, Department of Pathology (described in online Supporting Information Methods).

Skeletal muscle lipidomics

Untargeted lipidomic analysis was completed by the Michigan Regional Comprehensive Metabolomics Resource Core (described in online Supporting Information Methods).

Statistical analysis

All data were tested for normality (Shapiro–Wilk test) and log-transformed for non-normal distributions. For multivariate analyses, net elastic regression (Lasso) was used to subset clinical and deep-phenotyping factors associated with insulin-mediated glucose uptake using *glmnet* package in R (R, Vienna, Austria). Univariate analysis independently correlating all measured variables and insulin-mediated glucose uptake was analyzed by Pearson correlations and adjusted for age and sex using *psych* package. Our univariate correlations were also corrected for multiple comparisons using the Benjamini-Hochberg method [26] and are represented as *p*.adjust in *Results*. Student *t* tests compared metabolic outcomes in HS versus LS. For proteomic analysis, differential abundance was analyzed using *LIMMA*. Proteins with $p < 0.05$ and abundance ratio > 1.5 between groups were considered significantly different in abundance. For lipidomic analysis, lipids either negatively or positively associated with both insulin-mediated glucose uptake and FA Ra suppression were displayed based on significance adjusted for multiple comparisons [26]. β coefficients for standardized abundance (*z* score) of individual lipid species with respect to acyl-chain length and unsaturation were correlated with standardized rates of insulin-mediated glucose uptake and FA Ra suppression (β_z). Correlations of β_z versus acyl-chain length and β_z versus unsaturation were correlated by Pearson. Statistical analysis was completed using R version 4.1.0.

RESULTS

Study participants

Participant characteristics are presented in Table 1. All participants had obesity but were normoglycemic (hemoglobin $A_{1c} < 5.7\%$). Despite being homogeneous for body composition and most clinical measures, insulin-mediated glucose uptake and lipid kinetics varied widely among the entire study population (Figure 1).

Clinical and deep-phenotyping factors associated with insulin-mediated glucose uptake

Multiple linear regression for clinical outcomes indicated that high-molecular-weight adiponectin, high-density lipoprotein cholesterol, and total adiponectin directly correlated with insulin-mediated glucose uptake (non-zero coefficients from Lasso regression), whereas BMI, plasma TAG, visceral fat area, and liver fat percentage inversely correlated with insulin-mediated glucose uptake (Figure 2A; Supporting Information Table S2 contains complete explanatory variable list). For deep-phenotyping outcomes, insulin-mediated FA Ra suppression (expressed as percent change from basal FA Ra), basal hepatic glucose production (glucose Ra), fat oxidation, insulin-mediated suppression of hepatic glucose production, and glycerol Ra during the clamp directly correlated with insulin-mediated glucose uptake (Figure 2B). In

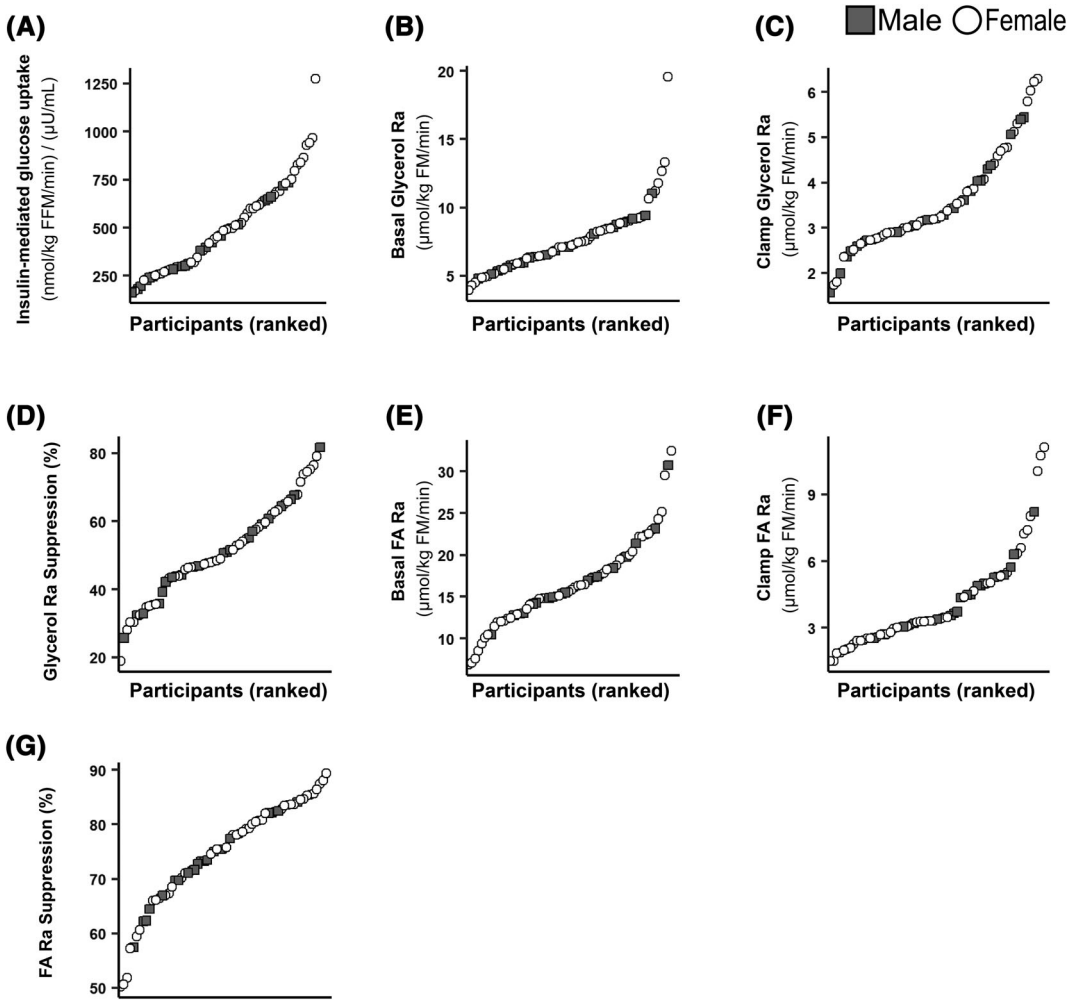

FIGURE 1 Insulin-mediated glucose uptake and lipid kinetics across all participants ($n = 66$). (A) Insulin-mediated glucose uptake. (B) Basal glycerol Ra. (C) Clamp glycerol Ra. (D) Insulin-mediated glycerol Ra suppression. (E) Basal FA Ra. (F) Clamp FA Ra. (G) Insulin-mediated FA Ra suppression. FA, fatty acid; FFM, fat-free mass; FM, fat mass; Ra, rate of appearance

contrast, FA Ra during the clamp inversely correlated with insulin-mediated glucose uptake (Figure 2B). The subset factors from the Lasso regression were then analyzed by multiple linear regression with the clinical factors model (MODEL 1) and a deep-phenotyping factors model (MODEL 2). Both models significantly associated with insulin-mediated glucose uptake (MODEL 1: adjusted $R^2 = 0.21$; $p < 0.01$; MODEL 2: adjusted $R^2 = 0.55$; $p < 0.01$; Supporting Information Table S3).

Univariate analysis indicated that insulin-mediated FA Ra suppression had the strongest positive relationship with insulin-mediated glucose uptake (Figure 2C,D). Importantly, it did not appear that FA Ra suppression was an artifact of variability in baseline or fasting FA Ra because the percent change in FA Ra in response to the insulin clamp was not associated with basal FA Ra ($r = 0.13$; $p = 0.28$). In line with the relationship between FA Ra suppression and insulin-mediated glucose uptake, FA Ra during the clamp ("clamp FA Ra") was negatively correlated with insulin-mediated glucose uptake when clamp FA Ra was normalized to fat mass (Figure 2C,E) and fat-free mass (Figure 2F).

Adipocyte cell size

Mean adipocyte area inversely associated with both insulin-mediated glucose uptake ($r = -0.29$; $p = 0.04$; Figure 3A) and FA Ra suppression ($r = -0.27$; $p = 0.05$; Figure 3B). However, after adjusting for multiple comparisons, statistical significance for these outputs was not maintained (p.adjust = 0.22 and 0.11, respectively). Using quartile stratifications to define small adipocytes (1000-3196 μm^2) and large adipocytes (>6784 μm^2), we found that the proportion of small adipocytes did not associate with insulin-mediated glucose uptake ($p = 0.18$, p.adjust = 0.18; Figure 3E) or FA Ra suppression ($p = 0.13$, p.adjust = 0.36; Figure 3F). However, although the correlation between the proportion of large adipocytes and insulin-mediated glucose uptake did not reach statistical significance (Figure 3G), the proportion of large adipocytes inversely correlated with FA Ra suppression ($r = -0.28$; $p = 0.04$; Figure 3H). This relationship did not quite reach statistical significance after adjusting for multiple comparisons (p.adjust = 0.098).

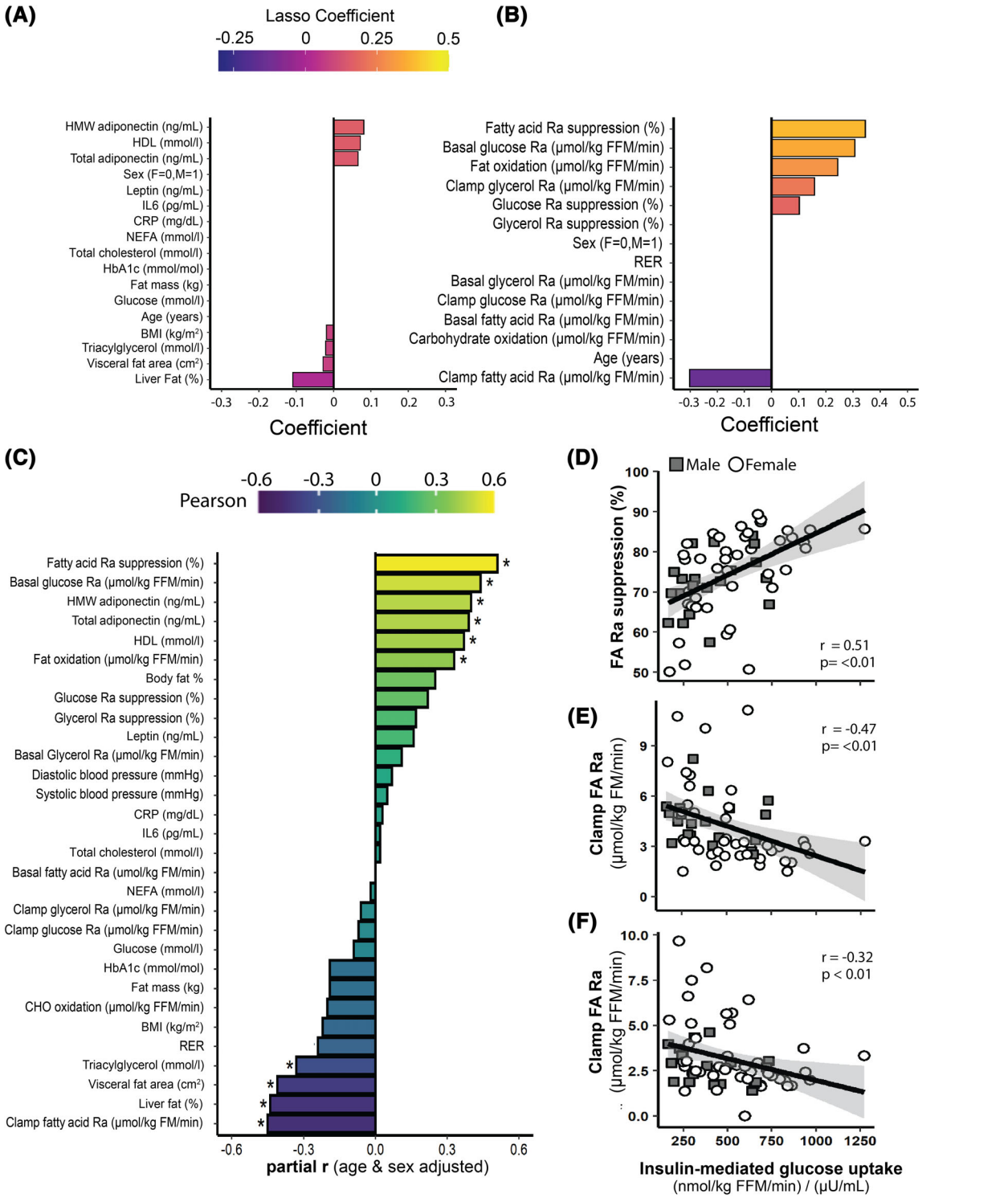

FIGURE 2 Clinical and deep-phenotyping factors associated with insulin-mediated glucose uptake. (A) Lasso regression coefficients for clinical factors associated with insulin-mediated glucose uptake. (B) Lasso regression coefficients for deep-phenotyping factors (i.e., substrate control and oxidation under fasting and hyperinsulinemia) and insulin-mediated glucose uptake. (C) Independent correlations (Pearson) for all individual factors associated with insulin-mediated glucose uptake, adjusted for age and sex (partial *r*). (D) Relationships among insulin-mediated glucose uptake and FA Ra suppression, (E) insulin-mediated FA Ra (clamp) normalized to fat mass, and (F) fat-free mass. *Significant correlation versus insulin-mediated glucose uptake ([nanomoles per kilogram of fat-free mass/minute]/[microunits/milliliter]) when adjusted for multiple comparisons (*p*.adjust < 0.05); $n = 66$. CRP, C-reactive protein; FA, fatty acid; FFM, fat-free mass; FM, fat mass; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; HMW, high molecular weight; NEFA, nonesterified fatty acid; Ra, rate of appearance; RER, respiratory exchange ratio

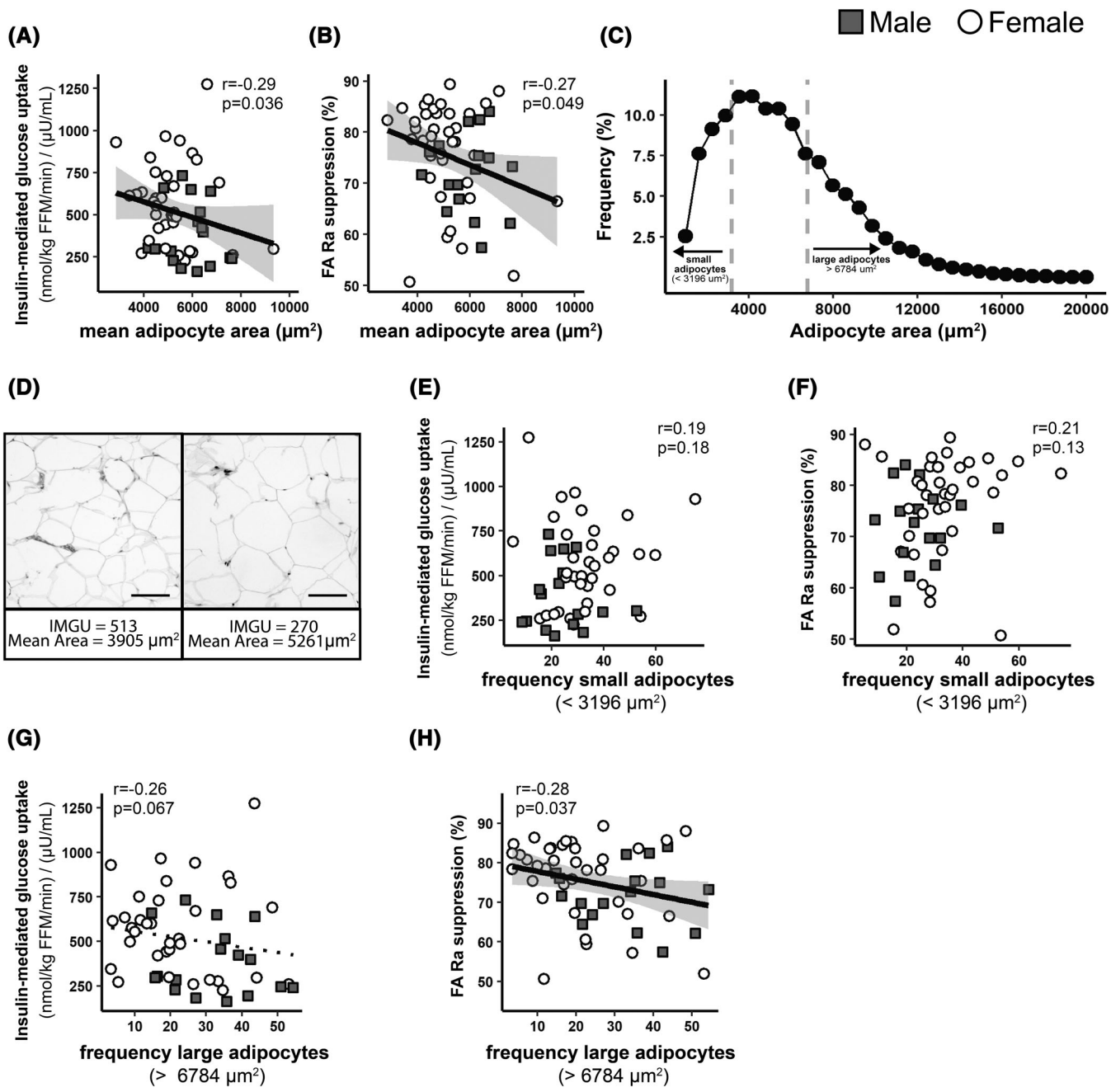

FIGURE 3 Relationships among adipose tissue cell size and insulin sensitivity. Association of mean adipocyte size with (A) insulin-mediated glucose uptake and (B) FA Ra suppression. (C) Adipocyte cell size distribution from study cohort (bins = 30). (D) Representative image for a participant with high (513 nmol/kg fat-free mass/min/[microunits/milliliter]) and low (270 nmol/kg fat-free mass/min/[microunits/milliliter]) insulin-mediated glucose uptake; scale bar = 100 µm. Association of the frequency of small adipocytes (1000-3196 µm^2) with (E) insulin-mediated glucose uptake and (F) FA Ra suppression. Association of the frequency of large adipocytes (>6784 µm^2) with (G) insulin-mediated glucose uptake and (H) FA Ra suppression; $n = 58$. FA, fatty acid; FA Ra, FA rate of appearance; FFM, fat-free mass

Subcohort stratification based on FA Ra suppression

Similar to the wide-ranging insulin-mediated glucose uptake rates among participants, insulin-mediated FA Ra suppression also varied widely (Figure 4A). FA Ra suppression in our participants in the HS group was ~45% greater than the LS group (HS = 82.5%-88.0% suppression; LS = 50.7%-68.5% suppression; Figure 4A). There were no differences in basal FA Ra between subcohorts; therefore, the difference in FA Ra suppression was consequential to differences in insulin-mediated FA Ra (Figure 4B). As anticipated, insulin-mediated glucose uptake was significantly greater in HS versus LS, whether normalized to plasma insulin concentration (Figure 4C) or not (Figure 4D). Glucose Ra suppression during the insulin clamp (hepatic insulin sensitivity index) was also greater in the HS group (Figure 4E,F). Although

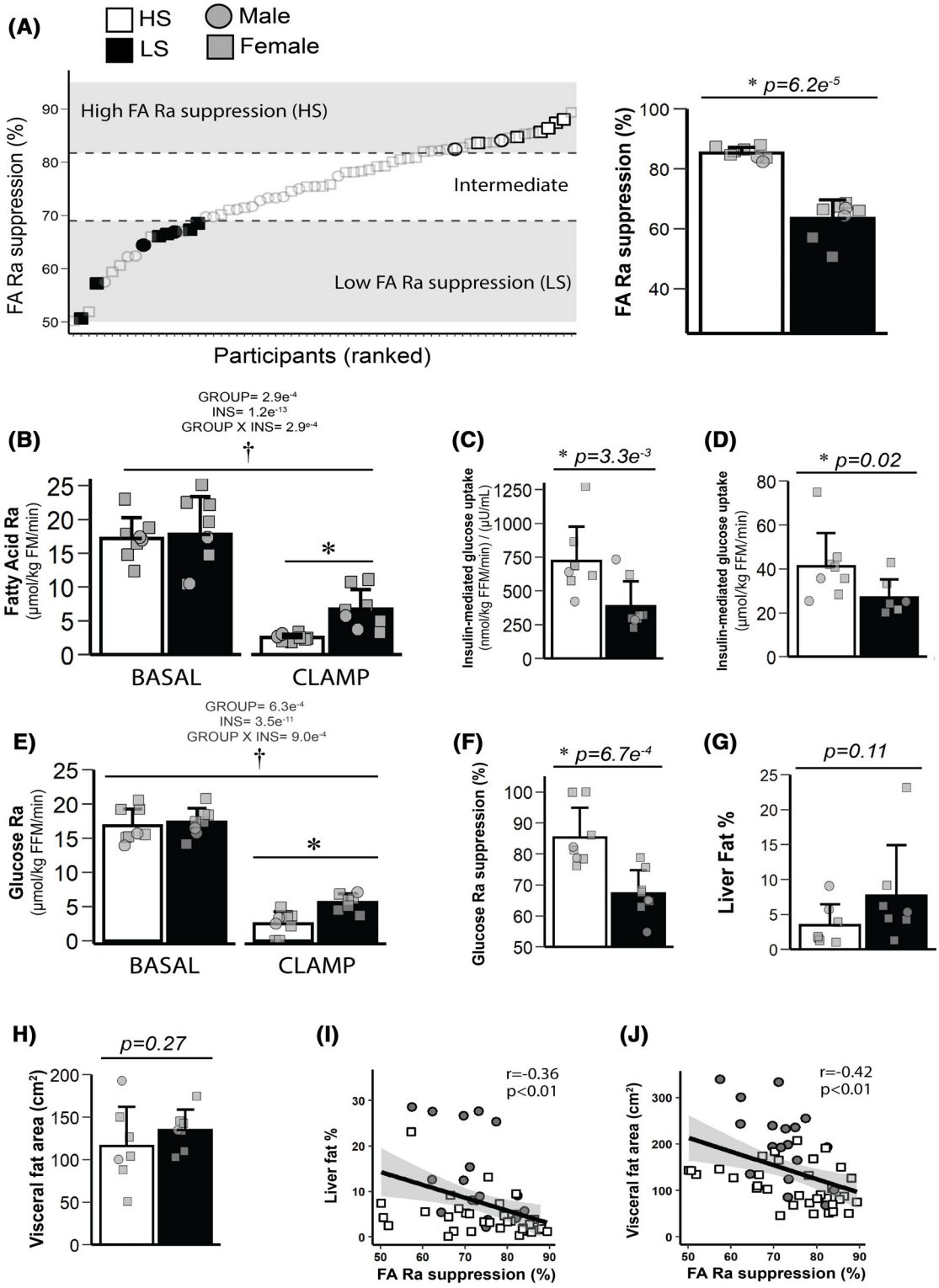

FIGURE 4 Comparison of insulin-mediated glucose uptake, hepatic insulin sensitivity, liver fat, and visceral fat area from a subcohort matched for body composition and discordant FA Ra suppression. (A) Participants who had discordant FA Ra suppression and were well matched for body composition and sex were paired into HS and LS subcohorts. (B) FA Ra measured under basal conditions and during the hyperinsulinemic-euglycemic clamp. (C) Measures of insulin-mediated glucose uptake normalized to insulin (nanomoles per kilogram of fat-free mass/minute/insulin) and (D) not normalized to insulin (micromoles per kilogram of fat-free mass/minute). (E) Glucose Ra measured before and during the hyperinsulinemic-euglycemic clamp. (F) Hepatic insulin sensitivity measured as glucose Ra suppression during the hyperinsulinemic-euglycemic clamp. (G) Liver fat percent and (H) visceral fat area (centimeters squared) among groups. (I) Association between liver fat percent and FA Ra suppression for all study participants. (J) Association between visceral fat area and FA Ra suppression for all study participants. Data are mean ± SD. $^{*}p < 0.05$, HS versus LS. $^{\dagger}p < 0.05$, basal versus clamp (insulin-stimulated) during hyperinsulinemic-euglycemic clamp; $n = 16$.
FA, fatty acid; FFM, fat-free mass; HS, high FA Ra suppression; LS, low FA Ra suppression; Ra, rate of appearance

there were no significant differences in liver fat or visceral fat between subcohorts (Figure 4G,H), when we examined this relationship across the entire study cohort, we found significant inverse correlations of FA Ra suppression with both liver fat and visceral fat ($p < 0.01$; Figure 4I,J).

aSAT proteomics

Untargeted proteomic analysis identified 2519 aSAT proteins (Figure 5A). Of the 2519 proteins identified, 148 proteins were differentially expressed in HS versus LS (115 proteins were greater in LS, whereas 33 were greater in HS; Figure 5B,C; Supporting Information Table S4).

ECM-receptor interaction was the highest significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enriched in the LS group (Figure 5D). Of proteins within ECM-receptor expression pathway, collagen proteins (COL1A1, COL3A1, COL23A1), a cytoskeleton binding protein (SEPT5), and integrin proteins (ITGA2B, ITGB3) were more abundant in LS versus HS (Figure 5E). In line with this finding, there was an inverse correlation between ECM fibrosis and insulin-mediated glucose uptake across the entire study cohort ($r = -0.37$; $p < 0.01$; Supporting Information Figure S2B). Although we found no significant differences between subcohorts for independent collagen VI isoforms (COL6A1-3), the sum of all COLVI isoforms ($\sum$COL6A1-3) was greater in LS versus HS ($p = 0.02$; Figure 5F). Additionally, transforming growth factor β1 (TGFβ1), a key mediator of fibrosis activated by inflammatory stimuli [27], was significantly greater in LS ($p = 1.18\ e^{-6}$; Figure 5G).

Skeletal muscle lipidomics

High rates of FA flux from aSAT into the systemic circulation contributes to greater FA uptake into skeletal muscle, accumulating bioactive lipids linked with skeletal muscle insulin resistance. Skeletal muscle lipidomics identified 734 lipid species across all participants, whereas 21 lipid species significantly correlated with both insulin-mediated glucose uptake and FA Ra suppression (Figure 6A,B; Supporting Information Table S5). Lipids that positively correlated with both insulin-mediated glucose uptake and FA Ra suppression consisted primarily of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) species, whereas lipids that inversely correlated with both insulin-mediated glucose uptake and FA Ra suppression included TAG, PC, plasmenyl-PE, and acylcarnitine (Figure 6C). When observing the relationship between insulin-mediated glucose uptake and intraclass lipid abundance, plasmenyl-PE abundance was inversely related with both insulin sensitivity and FA Ra suppression (Supporting Information Figure S3).

In an attempt to assess whether relationships existed among the conformation (i.e., acyl-chain length and saturation state) of key muscle lipids and insulin-mediated glucose uptake or FA Ra suppression, we plotted the β coefficient values for *z* score-transformed glucose uptake_z and FA Ra suppression_z versus the standardized abundance of muscle fatty acids (Figure 7A,B), acylcarnitines (Figure 7C,D), DAG (Figure 7E,F), and TAG (Figure 7G,H) for their respective acyl-chain length. Inverse correlations were found for insulin-mediated glucose uptake_z and acyl-chain length of skeletal muscle FA ($p = 0.04$, $p.\text{adjust} = 0.045$; Figure 7A), acylcarnitine ($p < 0.01$, $p.\text{adjust} < 0.01$; Figure 7C), and TAG ($r = -0.40$; $p < 0.01$, $p.\text{adjust} < 0.01$; Figure 7G). An inverse correlation between acylcarnitine chain length and FA Ra suppression_z was also found ($r = -0.42$; $p = 0.02$, $p.\text{adjust} = 0.03$; Figure 7D), whereas TAG chain length also inversely correlated with FA Ra suppression_z ($r = -0.51$; $p < 0.01$, $p.\text{adjust} < 0.01$; Figure 7H). The same analyses were performed to examine relationships among lipid class saturation with insulin-mediated glucose uptake_z and FA Ra suppression_z, and no significant relationships were present (Supporting Information Figure S4).

DISCUSSION

In agreement with work from our lab [3, 4] and others [5, 6], whole-body insulin-mediated glucose uptake varied considerably among a relatively homogeneous population of adults with obesity, and the rate of insulin-mediated suppression of FA release from aSAT may help explain this relationship. Because the majority of insulin-mediated glucose uptake occurs in skeletal muscle [11], our findings suggest that the ability for insulin to suppress FA release from aSAT may be an important mediator preserving skeletal muscle insulin sensitivity. Although our correlational analyses do not confirm causality, our findings are consistent with others [5, 6], as well as with findings demonstrating that pharmacological reduction in systemic FA mobilization improved insulin-mediated glucose uptake [16]. From a clinical perspective, we interpret our findings to suggest that enhanced insulin-mediated suppression of FA release may markedly lower FA release from aSAT after meals/snacks when insulin concentrations are high, thereby maintaining systemic FA availability at relatively low levels throughout the day and, in turn, limiting systemic FA availability for ectopic lipid accumulation in insulin-responsive tissues such as skeletal muscle and liver. Our current findings also expand on previous work that has suggested that aSAT morphology such as smaller adipocyte size and lower ECM fibrosis may contribute to enhanced insulin-mediated FA Ra suppression. Additionally, our novel findings suggest that greater insulin-mediated FA Ra suppression, which mitigates excessive systemic FA availability, may be contributing to a skeletal muscle lipid phenotype associated with enhanced insulin sensitivity. Overall, our findings suggest important tissue-specific relationships among adipose tissue with skeletal muscle and liver by which FA release from aSAT may have important implications on skeletal muscle and liver metabolism.

Excessive FA release from aSAT into the systemic circulation underlies many obesity-related cardiometabolic complications, including insulin resistance [1]. Because the majority of FA released into the circulation was shown to be derived from aSAT as opposed to visceral depots [7], the regulation of FA metabolism in aSAT is a major

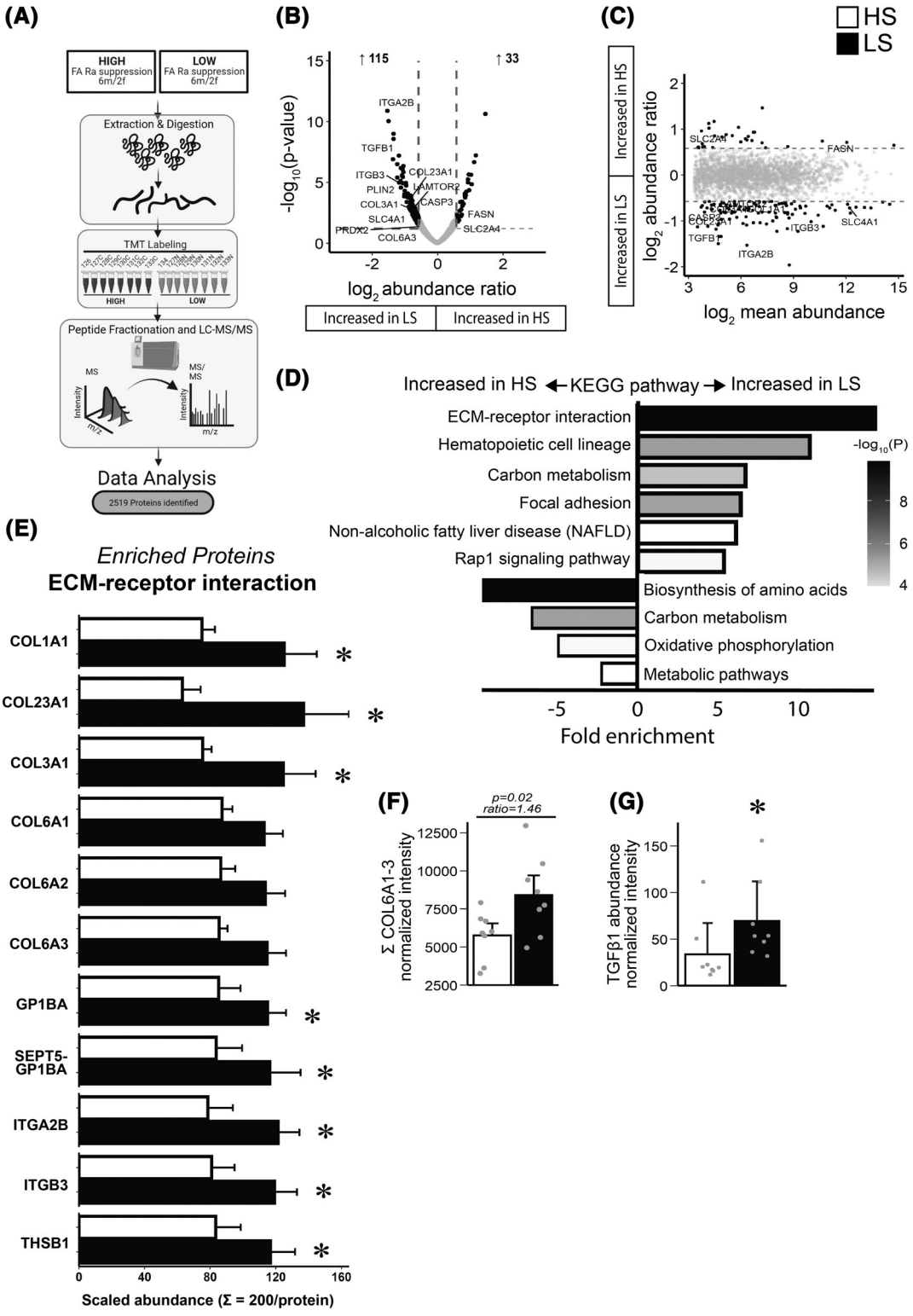

FIGURE 5 Comparison of adipose tissue proteome in HS versus LS subcohorts. (A) Overview of proteomic workflow. (B) Volcano plot showing differentially expressed proteins among groups ($p < 0.05$, abundance ratio > 1.5) and arrows indicating the number of proteins differentially expressed among groups. (C) MA plots demonstrating differentially expressed proteins with respect to integrative abundance. (D) KEGG pathway enrichment (top 10). (E) Differentially expressed proteins from ECM-receptor interaction KEGG pathway. Protein expression for (F) COL6A protein (∑COL6A1, COL6A2, COL6A3) and (G) TGFβ1. Data are mean ± SD. *$p < 0.05$ and abundance ratio ≥ 1.5; $n = 16$. ECM, extracellular matrix; FA, fatty acid; FA Ra, FA rate of appearance; HS, high FA Ra suppression; KEGG, Kyoto Encyclopedia of Genes and Genomes; LS, low FA Ra suppression

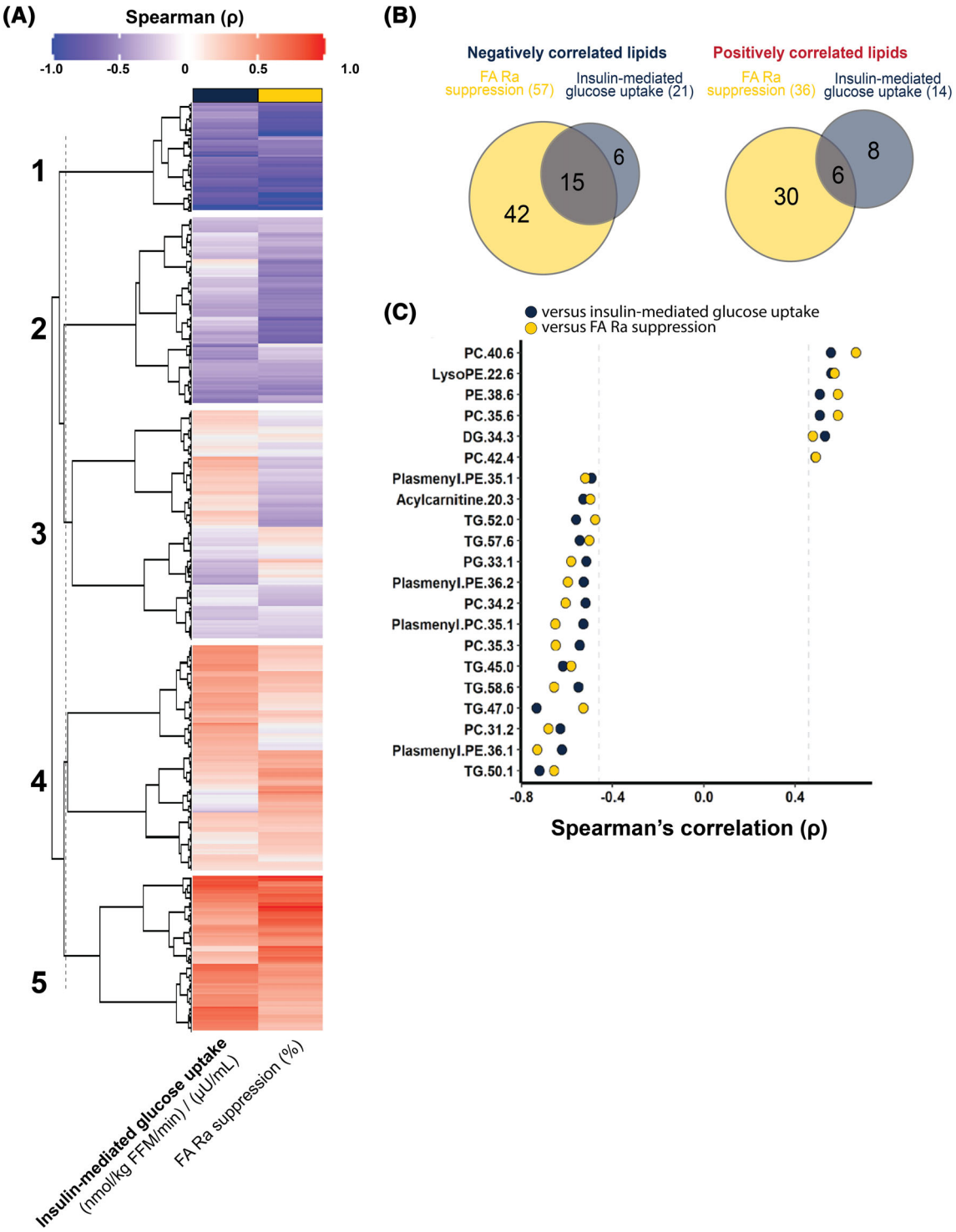

FIGURE 6 Relationships among insulin sensitivity and skeletal muscle lipidomic profile. (A) Hierarchical clustering heat map of lipid association with insulin-mediated glucose uptake and FA Ra suppression (k = 5). (B) Venn diagram of significant lipids grouped in cluster 1, negative association with both insulin-mediated glucose uptake and FA Ra suppression, and cluster 5, positive association with both insulin-mediated glucose uptake and FA Ra suppression. (C) Significant association of individual lipid species with both insulin-mediated glucose uptake and FA Ra suppression. Significant values adjusted for multiple comparisons; $n = 18$. FA, fatty acid; FA Ra, FA rate of appearance; FFM, fat-free mass

contributing factor for obesity-related complications. Our observation that, out of all parameters measured, insulin-mediated FA Ra suppression had the highest positive correlation with insulin-mediated glucose uptake supports important integrated effects of insulin on both aSAT and skeletal muscle, which is the primary site of insulin-mediated glucose uptake. Therefore, adults with obesity presenting

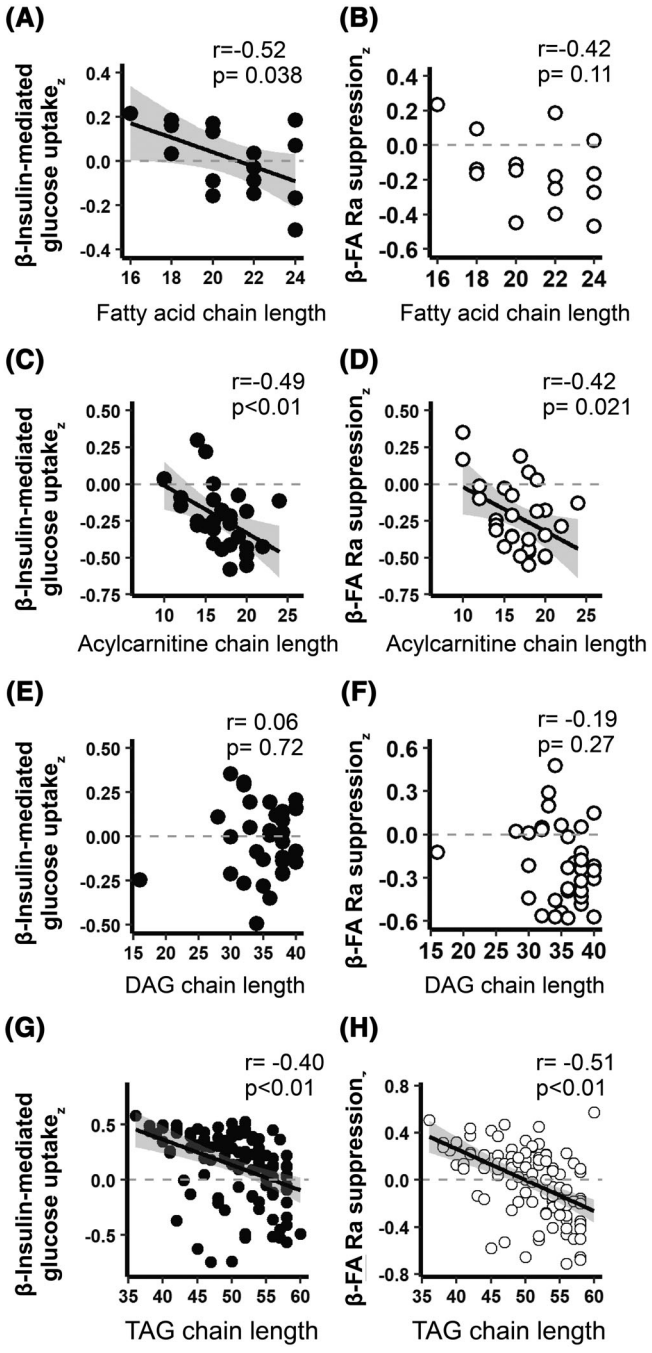

FIGURE 7 Relationships of β coefficients describing models with acyl-chain lengths of (A,B) skeletal muscle free FA, (C,D) acylcarnitine, (E,F) DAG, and (G,H) TAG on insulin-mediated glucose uptake$_z$ and FA Ra suppression$_z$. Individual data points represent each species within respective class of lipids; $n = 18$. DAG, diacylglycerol; FA, fatty acid; FA Ra, FA rate of appearance; TAG, triacylglycerol; glucose uptake$_z$, z score-transformed glucose uptake

with greater sensitivity to suppress insulin-mediated FA release from aSAT may be "protected" from skeletal muscle insulin resistance.

Insulin was shown to suppress FA release from adipose tissue by both inhibiting lipolysis and stimulating FA reesterification [28], thereby preventing FA liberated by lipolysis from leaving the adipocyte. Glycerol Ra data from this study (gold-standard measurement for whole-body lipolysis) suggest that differences in FA Ra suppression among participants were not due to differences in sensitivity to the antilipolytic effects of insulin, although we recognize that the relatively high insulin infusion rate may have near-maximally suppressed glycerol Ra in some individuals. Importantly, differences in FA reesterification may help explain the variability in FA Ra suppression, which was previously reported [29]. Intracellular FA reesterification is largely regulated by the acyltransferase proteins glycerol 3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT), and enhanced sensitivity of these proteins in response to insulin may contribute to greater FA Ra suppression. Interestingly, whereas DGAT activity may be insulin-responsive [30], the effect of insulin on GPAT activity is less clear [31]. In addition to the regulation of reesterification, factors such as aSAT blood flow and morphological features such as adipocyte size and fibrosis may also contribute to insulin-mediated FA Ra suppression variability [9, 32].

Much of the excess body fat mass in obesity is stored within hypertrophied adipocytes, and the abundance of large adipocytes was shown to be an important predictor of insulin resistance and type 2 diabetes [32]. FA release from isolated adipocytes *in vitro* was found to be greater in large adipocytes [33], which aligns with our findings that adipocyte area may be inversely associated with insulin-mediated FA Ra suppression. Hypertrophic adipocytes often present with elevated proinflammatory macrophage accumulation and cytokine release [34], which, in turn, may attenuate adipose tissue insulin signaling [35] and contribute to lower insulin-mediated FA Ra suppression. Additionally, lower capillary density in hypertrophied adipocytes, and a resultant compromise in microcirculation, may lower insulin delivery and blunt insulin-mediated FA Ra suppression *in vivo*.

Highly fibrotic aSAT ECM can also pose physical restrictions for adipocyte expansion during weight gain, and the resulting mechanical stress within the adipocyte was proposed to increase proinflammatory cytokine production [36]. Conversely, the reduction of adipose tissue ECM fibrosis in collagen VI$^{-/-}$ mice was found to markedly reduce inflammatory insult in adipose tissue and vastly improve overall metabolic health [37]. Preclinical models have also demonstrated that adipose tissue fibrosis attenuates insulin signaling in adipose tissue [37, 38]. In humans, the influence for ECM fibrosis to modify insulin signaling or insulin delivery to the adipocyte has been speculated to modify FA mobilization but has not been confirmed, to our knowledge. In the current study, our observation that participants in the LS subcohort had greater collagen accumulation supports the prospect that fibrotic aSAT ECM may negatively influence insulin-mediated lipid metabolism.

Interestingly, adipose tissue fibrosis appears to be both a cause and consequence of increased inflammatory stress within the tissue [9, 39]. TGFβ1 is implicated in fibrosis development and derived as a result of proinflammatory stimuli [39, 40]. Activation of TGFβ1 is responsible for progenitor cell differentiation into myofibroblasts and it may contribute to increased progenitor cell differentiation away from adipogenesis toward myofibroblast differentiation in ECM [27, 40]. Our finding that greater collagen abundance in the LS subcohort

was accompanied by elevated TGFβ1 aligns with the important role of TGFβ1 in fibrogenesis. Some of the increased inflammatory stimuli that may activate TGFβ1 in adipose tissue can stem from hypoxia induced by low tissue perfusion of hypertrophied adipocytes. Additionally, local hypoxia may increase fibrosis independently of TGFβ1 through hypoxia-inducible factor 1-α (HIF1α)-mediated activation of platelet-derived growth factor (PDGF) receptor β progenitor cells toward a myofibroblast lineage [41, 42]. Together, hypoxia and inflammatory stimuli may be important factors promoting aSAT ECM fibrosis and they may contribute to impaired insulin-mediated FA Ra suppression in aSAT.

Perhaps the most important clinical implication of retaining high insulin-mediated FA Ra suppression is the potential to limit ectopic lipid accumulation. Chronically elevated FA delivery and uptake into liver can result in hepatic steatosis and related disorders, which can greatly impair hepatic glucose metabolism and insulin sensitivity [43]. Importantly, our findings that hepatic insulin sensitivity was significantly greater in HS and that liver fat was inversely correlated with FA Ra suppression across all participants support the favorable impact of enhanced insulin-mediated FA Ra suppression on liver metabolism [43]. Additionally, although visceral fat accumulation is commonly linked to cardiometabolic complications, much of the fat stored in visceral fat is derived from FA released from aSAT [7]. Our finding that visceral fat area inversely correlated with insulin-mediated FA Ra suppression suggests the possibility that this effect may diminish visceral fat, thereby reducing health risks associated with excess visceral fat accumulation.

The link between excessive skeletal muscle FA uptake and insulin resistance has been largely attributed to excess lipid intermediate and metabolite accumulation, such as DAG [12], ceramide [13], and long-chain acyl-CoA [14, 15]. Although we did not find skeletal muscle DAG or ceramide to be associated with insulin-mediated glucose uptake, our findings that acyl-chain length of FA, acylcarnitines, and TAG in skeletal muscle was inversely related with insulin-mediated glucose uptake provide indirect evidence to suggest that aberrant lipid metabolism may contribute to impaired skeletal muscle insulin action. These findings are, in general, in agreement with a previous study demonstrating that lowering 24-hour FA availability with acipimox (potent lipolytic inhibitor) decreased long-chain fatty acyl-CoA accumulation in skeletal muscle with an accompanying improvement in insulin sensitivity [16]. Long-chain lipid accumulation in skeletal muscle can be a consequence of "incomplete" FA oxidation due to high FA uptake rates and β oxidation relative to flux through the tricarboxylic acid cycle [15], which is very low when sedentary. Although TAG is a neutral lipid, we found that long-chain TAG accumulation was inversely related with whole-body insulin sensitivity, but the implications of this finding are not clear.

An important limitation of this clinical study is its high reliance on correlational analyses, which hinders our ability to assign causation to integrative interpretations. For example, we suggest that impaired insulin-mediated FA Ra suppression from aSAT may precede insulin resistance for glucose metabolism in muscle and liver, but we acknowledge that this interpretation is based on correlations. Additionally, although we address the potential role of hypoxia and inflammation on fibrogenesis in aSAT, this supposition was based on prior evidence [41, 42, 44], and direct data to support this notion are not provided. We also acknowledge several other systemic mechanisms may be contributing to low insulin-mediated FA Ra suppression, including sympathetic tone, cellular heterogeneity, and senescence, and may be implicated in aSAT dysfunction. Last, although the participants in our LS and HS subcohorts were sex-matched, we recognize that sex differences are important contributing factors to differences observed throughout the entire study cohort.

In summary, our findings indicate that greater insulin-mediated suppression of FA release from aSAT may preserve insulin-mediated glucose uptake, hepatic insulin sensitivity, and lower liver and visceral fat (Supporting Information Figure S5). Together, these findings support the prospect that sustaining the ability for insulin to potently suppress FA release from aSAT may be critical for maintaining metabolic health in adults with obesity. Adipocyte size, aSAT inflammation, and fibrosis may be important mediators of aSAT insulin response. These proposed effects in aSAT may contribute to lower insulin-mediated glucose uptake, perhaps in part through the accumulation of long-chain FA, acylcarnitine, and TAG. Overall, our findings point to an important tissue-specific cross talk by which retaining insulin response to suppress FA release from aSAT has impactful metabolic implications in other tissues.

AUTHOR CONTRIBUTIONS

Michael W. Schleh, Benjamin J. Ryan, Cheehoon Ahn, Alison C. Ludzki, Jenna B. Gillen, Douglas W. Van Pelt, Lisa M. Pitchford, Thomas Rode, and Jeffrey F. Horowitz contributed to study design and performed data acquisition. Suzette M. Howton coordinated participant recruitment. Michael W. Schleh and Jeffrey F. Horowitz performed data analysis and interpretation of the data. Michael W. Schleh and Jeffrey F. Horowitz drafted the manuscript. All authors read and revised the final version of the article to be published and have provided final approval.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The source data and R code used for clinical assessment analysis, adipose tissue proteomics, and lipidomics are available upon request with the corresponding authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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