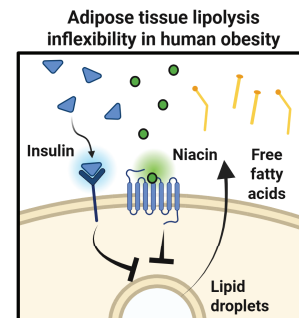
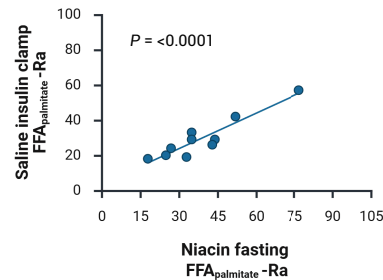
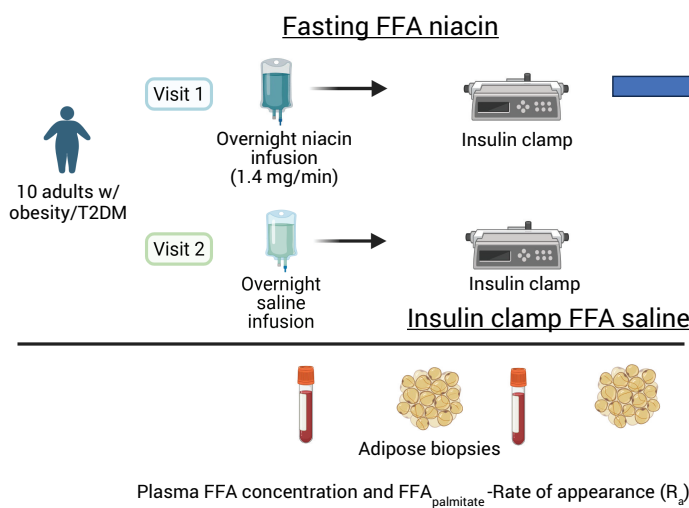


Adipose Tissue Resistance to the Antilipolytic Effect of Insulin and Niacin in Humans With Obesity

Shuhao Lin, Kelli A. Lytle, Nicola Fink, and Michael D. Jensen

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The ability of insulin and niacin to suppress lipolysis is strongly correlated in adults with obesity/T2DM.



Dysregulated adipose tissue lipolysis in obesity/T2DM is likely due to defective responses of distal lipolysis proteins rather than isolated adipocyte insulin resistance.



Adipose Tissue Resistance to the Antilipolytic Effect of Insulin and Niacin in Humans With Obesity

Shuhao Lin,¹ Kelli A. Lytle,¹ Nicola Fink,² and Michael D. Jensen¹

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Adipose tissue (AT) lipolysis insulin resistance results in excess free fatty acid (FFA) release. We tested the hypothesis that the ability of insulin to suppress AT lipolysis is unrelated to the ability of niacin to suppress lipolysis, because niacin acts through a different proximal signaling pathway. Ten volunteers (5 women/5 men) with upper body obesity and/or type 2 diabetes mellitus (T2DM) underwent two study visits with overnight intravenous infusions of niacin (1.4 mg/min) or saline, followed by a hyperinsulinemic-euglycemic clamp. FFA-palmitate R_a was measured using [U-¹³C] and [²H₉]palmitate infusions; abdominal AT biopsies were performed before and during the insulin clamp. The suppression of FFA-palmitate R_a by insulin on the saline control day and by niacin after an overnight infusion were highly correlated ($r = -0.93$, $P < 0.001$). Fasting AT Akt (pAkt^{S473/474}-to-panAkt ratio, $P = 0.01$) and perilipin 1 (PLN 1) (pPLN1^{S552}-to-panPLN1 ratio, $P = 0.02$) phosphorylation were less during niacin than the saline control study. Because the suppression of lipolysis by insulin and niacin are highly correlated within individuals and because niacin and insulin act through different proximal signaling pathways, we propose dysregulated AT lipolysis in obesity/T2DM is due to dysfunction(s) in distal lipolysis proteins rather than isolated “insulin resistance.”

Abnormally elevated plasma free fatty acid (FFA) concentrations, arising from dysfunctional AT in the context of obesity, are implicated in the pathogenesis of insulin resistance and type 2 diabetes mellitus (T2DM). Adipose tissue (AT) lipolysis, the process by which FFA are liberated from intracellular triglycerides, is normally exquisitely sensitive to the

ARTICLE HIGHLIGHTS

- We undertook this study to compare adipose tissue lipolysis responses to insulin and niacin in humans.
- We tested the hypothesis that adipose tissue insulin resistance would be unrelated to adipose tissue niacin resistance.
- The suppression of lipolysis by insulin and niacin were highly correlated.
- Dysregulated adipose tissue lipolysis in obesity/type 2 diabetes is due to dysfunction(s) in distal lipolysis proteins rather than isolated “insulin resistance.”

suppressive effects of insulin (1). People with an upper body obesity (UBO) phenotype and/or T2DM have impaired AT responses to insulin, resulting in greater plasma FFA concentrations than those who are lean and people with a lower body obese phenotype (2,3). However, the exact mechanism behind such phenomenon remains unclear.

Niacin (e.g., nicotinic acid [NA]), when administered in pharmacological doses, is able to suppress AT lipolysis and thereby reduce plasma FFA concentrations (4). The intracellular signaling pathway that mediates the antilipolytic effect of niacin is not well understood. Although it no doubt must inhibit the activity of some of the proteins more directly involved in the hydrolysis of triglycerides, niacin has been shown to suppress lipolysis independent of the canonical proximal insulin signaling pathway (5,6).

We hypothesized that if AT insulin resistance is specifically located in the proximal canonical insulin signaling

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pathway, the responses to the antilipolytic effects of niacin would remain intact in people with adipose insulin resistance. To test this hypothesis, we quantitated the effects of insulin and niacin on lipolysis suppression in adults recruited to have a wide range of insulin action with respect to suppressing AT lipolysis. To accomplish this, we administered an overnight infusion of intravenous niacin on one study day and overnight intravenous saline on a second study day, followed by a euglycemic-hyperinsulinemic clamp on each day. This allowed us to measure the ability of both niacin and insulin to suppress FFA release. To further understand the cellular responses, we measured changes in the phosphorylation of AT protein kinase B (Akt) and lipolysis proteins.

RESEARCH DESIGN AND METHODS

This protocol had two major goals. One was to examine the ability of niacin to suppress lipolysis relative to insulin in volunteers selected to have a wide range of adipose insulin responsiveness. The other was to investigate the effects of overnight intravenous niacin on muscle responses to insulin. We used two different palmitate tracers and conducted insulin clamps during both the niacin study day and saline control study day (as described below) to investigate the effect of niacin infusion on muscle responses of insulin, including the trafficking of FFA-palmitate into muscle lipids, using a “pulse/chase” study design. The muscle data will be reported elsewhere. The Mayo Clinic Institutional Review Board (Rochester, MN) approved the study, and all participants provided written informed consent.

Study Participants

Ten Caucasian volunteers, including five premenopausal women and five men with UBO and/or T2DM, completed this study. The volunteers were between 18 and 65 years old and were weight stable for at least 2 months prior to the study. Baseline laboratory screening was performed to ensure platelets count $>100,000/\text{dL}$, hemoglobin $\geq 12\text{g}/\text{dL}$ for men and $\geq 11.0\text{ g}/\text{dL}$ for women, and that women were not pregnant or nursing. Entry criteria included BMI between 29.0 and 40.0 kg/m^2 and a waist-to-hip ratio >0.85 for women and >0.95 for man. We included three participants with T2DM; inclusion criteria were an HbA_{1c} between 6.5 and 11.5% and not treated with pioglitazone or insulin. Exclusion criteria were a history of ischemic heart diseases, atherosclerotic valvular diseases, uncontrolled hypertension despite medication, smokers, allergic to lidocaine, niacin, or Niaspan, or taking medications that can alter serum lipid profiles (i.e., statins, β -blockers, etc.). The inclusion of both adults with uncomplicated obesity and T2DM allowed us to include participants with a wide range of AT insulin resistance.

Study Design

A summary of the study design is shown in Fig. 1. This study included two admissions ≤ 2 months apart to the

Mayo Clinic Clinical Research and Trials Unit (CRTU). Each admission included an overnight stay with intravenous access, followed by a euglycemic-hyperinsulinemic clamp the next morning. For the first study visit, volunteers received an overnight, intravenous niacin infusion, and for the second (control) visit, they received an intravenous saline infusion. Prior to each study, participants received their meals from the CRTU Metabolic Kitchen for 3 days to provide an isoenergetic diet (45% carbohydrate, 20% protein, and 35% fat). This ensured consistency of energy and nutrient intake. Participants were provided a standardized meal the evening of admission to the CRTU, and a forearm intravenous infusion catheter was placed, as was an intravenous hand vein catheter for blood sampling. Each volunteer received 325 mg of aspirin with the evening meal to reduce the likelihood of niacin-induced cutaneous flushing.

The overnight infusion of niacin (visit 1) or saline (visit 2) started at 2100 h. The starting dose of niacin was 0.6 mg/min, increasing 1.0 mg/min after 15 min if tolerated and to the goal rate of 1.4 mg/min after 30 min, again if tolerated; only one participant experienced a mild sensation of flushing that lasted <10 min and did not prevent dose escalation. Plasma samples were drawn at regular intervals overnight for measurement of plasma FFA concentrations and niacin metabolites (in a subgroup of volunteers). At 0400 h, an infusion of $[\text{U-}^{13}\text{C}]$ palmitate (330 nmol/min) was initiated to measure palmitate kinetics. Between 0830 h and 0900 h, a series of baseline blood samples (quadruplicates) were drawn in 10-min intervals to measure plasma FFA concentrations, palmitate enrichment, as well as plasma insulin and niacin metabolite concentrations. Immediately thereafter we performed a subcutaneous abdominal adipose biopsy with a small liposuction cannula after infiltration of the tissue with diluted local anesthetic.

After the blood sampling and AT biopsy were completed, we 1) discontinued the $[\text{U-}^{13}\text{C}]$ palmitate infusion; 2) started a continuous infusion of $[\text{H}_2]$ palmitate (400 nmol/min); 3) started a primed, continuous infusion of insulin ($1\text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) with 50% dextrose to maintain plasma glucose between 90 and 95 mg/dL; and 4) reduced the niacin infusion rate to 0.35 mg/min. Arterialized venous plasma samples were collected every 10 min to measure plasma glucose (YSI, Yellow Springs, OH) to help adjust the glucose infusion rate. Over the last 30 min of the 5-h insulin clamp, we collected quadruplicate blood samples, followed by a second abdominal adipose biopsy contralateral to the first biopsy.

Plasma Analysis

We measured plasma concentrations of hormones and catecholamines known to regulate lipolysis. Plasma samples were stored at -80°C until analyzed. Plasma insulin and serum growth hormone concentrations were measured by the Mayo Clinic Immunochemical Core Lab through an automated immunoassay. Plasma epinephrine

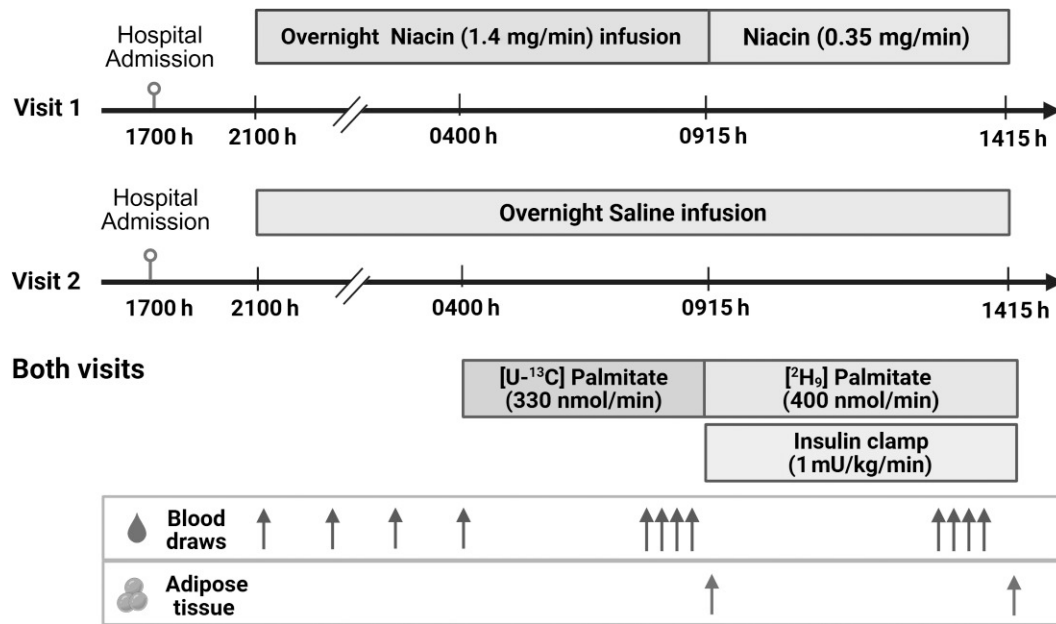


Figure 1—Study design. Figure was created in BioRender, <https://BioRender.com/ga7hfi7>.

and norepinephrine were measured by Mayo Clinic Central Laboratories. Concentrations of plasma NA and two primary NA metabolites, nicotinic acid (NUA) and nicotinamide (NAM), were measured by liquid chromatography–tandem mass spectrometry (Agilent Technologies, Santa Clara, CA) in positive ion mode using methods adopted from Pilli et al. (7). In brief, an 11 calibration curve was created for each substrate with the following range: 0–640 ng/ μ L for NA, 0–320 ng/ μ L for NAM, and 0–40 ng/ μ L for NUA. Then, 50 μ L of a mixture of stable isotope-labeled internal standards (NA- $^{13}\text{C}_6$ (1 ng/ μ L), NAM- $^{13}\text{C}_6$ (1 ng/ μ L), NUA-d4 (0.25 ng/ μ L), and 20 μ L formic acid were added to the calibration curve and 200 μ L plasma samples before extraction. Samples were extracted using 5 mL ethyl acetate as described (7), with the addition of 1% ammonium bicarbonate and chloroform to reduce ion suppression. Multiple reaction monitoring was used to monitor the precursor/product ions at 124/80.1 charge/mass ratio (m/z) for NA, 123/80.1 m/z for NAM, 180.9/134.9 m/z for NUA, 130.1/85.2 m/z for NA- $^{13}\text{C}_6$, 129.1/85.2 m/z for NAM- $^{13}\text{C}_6$, and 185/139.2 m/z for NUA-d4. The data were analyzed using Agilent MassHunter Quantitative Analysis software. These plasma metabolites were measured on samples collected from six of our participants. Plasma fatty acid concentrations and enrichment of [U- ^{13}C]palmitate and [$^2\text{H}_9$]palmitate, were measured using liquid chromatography/mass spectrometry as previously described (8).

AT Analysis

AT was rinsed with 0.9% NaCl immediately after collection, and any visible connective tissue and blood clots were removed. Adipocytes were isolated after collagenase

digestion, and adipocyte cell size was measured (9) on both study days; the sizes were not different ($P = 0.26$) between the two study days. An aliquot of AT was immediately flash frozen for measurement of intracellular AT proteins. Capillary Western blot technology (10) was used to measure adipose protein kinase B (Akt) (clone C67E7, Cell Signaling) and Akt phosphorylation (pAkt $^{S473/474}$) (clone D9E, Cell Signaling), perilipin-1 (PLN1) (70R-1297, Fitzgerald Industries International), and PLN1 phosphorylation (pPLN1 S552) (4856, Vala Sciences), as well as hormone-sensitive lipase (HSL) (Cell Signaling, no. 4107) and HSL phosphorylation (pHSL S660) (Cell Signaling, no. 45804). Data are expressed as the ratio of phosphorylated protein versus total protein content.

Calculations

We used palmitate R_a , calculated using a steady-state kinetic formula (11), as our measure of AT lipolysis. Measurements collected during 0830 h and 0900 h are referred to as “fasting” measurements, and measurements collected during the last 30 min of the insulin clamp are referred to as “clamp” in our Results section. The ability of niacin to regulate palmitate R_a was calculated as the percent suppression of the fasting palmitate R_a values on the niacin visit relative to the saline control day. The ability of insulin to regulate palmitate R_a was calculated as the percentage suppression of palmitate R_a during the clamp relative to fasting on the saline control day.

Statistical Analysis

Descriptive data are expressed as mean \pm SD, unless noted otherwise. The Shapiro-Wilk test was used to test for normality. Our primary null hypothesis was that there

Table 1—Baseline characteristics

| Participant characteristics | |
|--------------------------------------|-------------|
| (N = 10) | |
| Demographics | |
| Age (years) | 38 ± 10 |
| Sex (male/female) | 5/5 |
| Height (cm) | 175 ± 10 |
| Weight (kg) | 101.8 ± 8.7 |
| BMI (kg/m ²) | 33.5 ± 3.1 |
| Body composition | |
| Body fat (%) | 42 ± 7 |
| Fat mass (kg) | 40.4 ± 6.8 |
| Lean mass (kg) | 57.3 ± 9.1 |
| Visceral fat area (cm ³) | 161 ± 62 |
| Visceral fat (kg) | 5.2 ± 1.8 |
| Upper body subcutaneous fat (kg) | 22.4 ± 4.9 |
| AT characteristics | |
| Abdominal fat cell size (μg/cell) | 0.99 ± 0.17 |
| Cardiometabolic risk factors | |
| Fasting glucose (mg/dL) | 109 ± 35 |
| Total cholesterol (mg/dL) | 163 ± 33 |
| LDL cholesterol (mg/dL) | 94 ± 28 |
| HDL cholesterol (mg/dL) | 46 ± 11 |
| Triglycerides (mg/dL) | 115 ± 46 |

Data are expressed as mean ± SD or *n*. Abdominal adipocyte size is from the saline control study day.

would be no relationship between the antilipolytic response to insulin on the saline control study day and the overnight, fasting antilipolytic response to niacin. The Pearson correlation coefficient (*r*) was used to measure associations between palmitate R_a during niacin infusion and palmitate R_a during insulin clamp on saline day. A two-tailed *P* value of <0.05 was considered significant. Between-treatment data were analyzed using paired-sample *t* test. Data were analyzed using SPSS 28.0 software (IBM, Armonk, NY).

Data and Resource Availability

The data described in the manuscript, code book, and analytic code will be made available pending request from Dr. Michael Jensen (jensen@mayo.edu).

RESULTS

Participant characteristics are in Table 1. Three volunteers with T2DM participated in these studies. Two of these volunteers were prescribed metformin, and one was prescribed sulfonylurea for treatment; their HbA_{1c} was 7.1 ± 1.7%.

Plasma Concentrations of Niacin, Niacin Metabolites, and FFAs

We measured plasma niacin and niacin metabolites in six of our volunteers (Fig. 2A). Plasma NA concentrations achieved steady state, averaging of >600 ng/mL, within ~1 h of beginning the infusion, whereas plasma NAM

concentrations rose steadily for ~8 h. Plasma NA concentrations decreased rapidly after the niacin infusion rate was reduced, while plasma NAM concentrations slowly decreased.

The plasma FFA concentrations observed during the overnight niacin and overnight saline infusions, as well as during the insulin clamp, are depicted in Fig. 2B and given in Table 2. Plasma FFA concentrations were significantly suppressed during the overnight niacin infusion. We found no relationship between the plasma NA concentrations and the percentage suppression of overnight fasting (baseline) palmitate concentrations or palmitate-FFA R_a compared with saline control day. This is consistent with the observation that the dose of intravenous niacin we infused achieves concentrations at or above those needed to for maximal, achievable suppression of lipolysis (12). There was no statistical difference in plasma FFA concentrations over the last 30 min of the insulin clamp between saline and niacin visit days.

Insulin and Niacin Suppression of Lipolysis

The insulin infusion rate we used (1 mU · kg⁻¹ · min⁻¹) attained concentrations (Table 2) that achieved nearly complete antilipolytic activity (13). Palmitate-FFA R_a was suppressed by 65% by insulin on the saline control study day (Fig. 3A). Consistent with our goal, there was a wide range (50–80%) of palmitate-FFA R_a suppression by insulin.

Next, we compared palmitate kinetics between niacin and saline visits. Although fasting (preinsulin clamp) palmitate R_a was significantly less during the niacin infusion than the saline infusion (Table 2), palmitate R_a at the end of the insulin clamp was not different between the two study days (Table 2). Figure 3B depicts individual baseline palmitate release rates, respectively, after the overnight saline infusion and the overnight niacin infusion study visits. Compared with the saline control study day, the intravenous niacin infusion suppressed fasting palmitate-FFA R_a by an average of 55%. As with the response to insulin, we observed substantial interindividual variability (range 31–73%) in maximal niacin suppression of palmitate-FFA R_a . Moreover, we found that the fasting palmitate-FFA R_a measured during the niacin infusion was highly correlated (*r* = 0.93, *P* < 0.001) with the palmitate-FFA R_a measured during the insulin clamp on the saline control study day (Fig. 3C). A similar correlation (*r* = 0.85, *P* = 0.002) was found when results were expressed in percentage suppression of palmitate-FFA R_a by niacin and insulin, taking baseline palmitate-FFA R_a into account. (Fig. 3D)

Plasma Hormone and Catecholamine Concentrations

The plasma insulin concentrations observed under fasting and insulin clamp conditions were not different between the two study days (Table 2), whereas plasma growth hormone concentrations were greater under niacin than saline control conditions. Plasma epinephrine concentrations

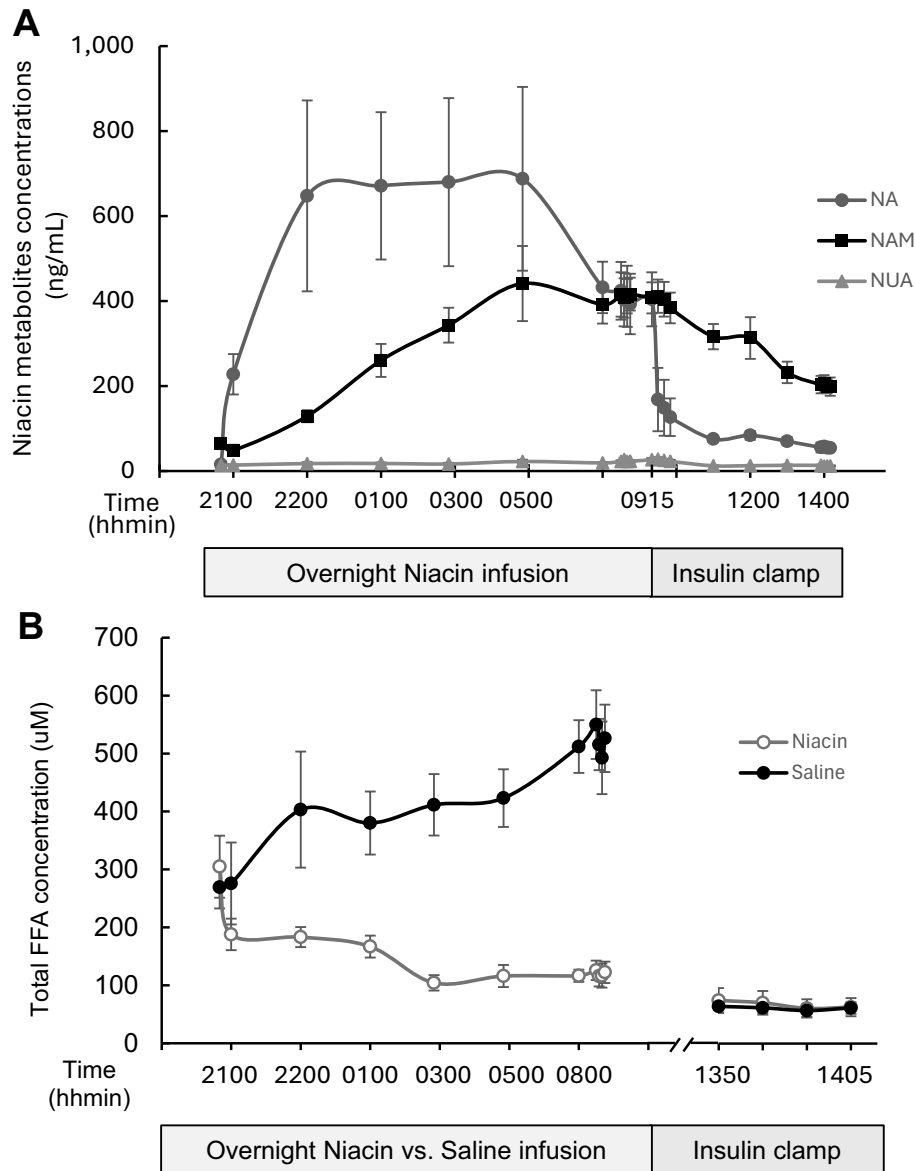


Figure 2—A: Plasma concentration of niacin metabolites on the study day with the overnight niacin infusion for six volunteers. B: Plasma concentration of total FFA on both the niacin and saline infusion study days for all 10 volunteers. Data expressed as mean concentrations. Error bars represent SE.

were not different between fasting and insulin clamp conditions or between niacin and saline conditions. Plasma norepinephrine concentrations were greater under insulin clamp than fasting conditions on the saline day, but not the niacin day.

AT Protein Responses to Insulin and Niacin

Intracellular AT protein data are depicted in Fig. 4. The pAkt^{S473/474}-to-panAkt and the pPLN1^{S552}-to-panPLN1 ratios were greater in fasting (baseline) adipose samples collected on the saline control day than on the niacin infusion study day ($P = 0.01$ and $P = 0.02$, respectively). However, there was no significant difference between the pAkt^{S473/474}-to-panAkt and the pPLN1^{S552}-to-panPLN1

ratios in the adipose biopsy samples collected during insulin clamp on the 2 study days. The AT pHSL^{S660}-to-panHSL ratios did not differ between fasting or insulin clamp biopsy samples between the saline and niacin study days.

DISCUSSION

This study is the first study to directly compare within-individual differences in the ability of intravenous niacin and intravenous insulin to suppress human AT lipolysis, as measured by FFA-palmitate release rates. We administered intravenous niacin overnight on one study day and saline on another study day to volunteers who also received FFA tracers and underwent an insulin clamp to

| | Saline | | Niacin | |
|---|----------------|-----------------|---------------|-----------------|
| | Fasting | Insulin clamp | Fasting | Insulin clamp |
| Plasma FFA | | | | |
| Total FFA concentration ($\mu\text{mol/L}$) | 521 \pm 158 | 52 \pm 25† | 120 \pm 54* | 56 \pm 47† |
| Palmitate R_a ($\mu\text{mol/min}$) | 84 \pm 17 | 30 \pm 11† | 39 \pm 16* | 37 \pm 25 |
| Catecholamines | | | | |
| Epinephrine (pg/mL) | 39 \pm 13 | 56 \pm 24 | 44 \pm 20 | 42 \pm 12 |
| Norepinephrine (pg/mL) | 186 \pm 71 | 216 \pm 49† | 183 \pm 48 | 189 \pm 65 |
| Hormones | | | | |
| Growth hormone (ng/mL) | 0.6 \pm 0.9* | 2.0 \pm 2.5 | 2.9 \pm 3.0 | 1.7 \pm 2.3 |
| Insulin ($\mu\text{IU/mL}$) | 7.5 \pm 2.7 | 60.4 \pm 11.1 | 6.0 \pm 3.2 | 60.9 \pm 10.5 |

Data expressed as mean \pm SD. Two-tailed P value by paired sample t test. † $P < 0.05$ between fasting and insulin clamp. * $P < 0.05$ between niacin and saline day.

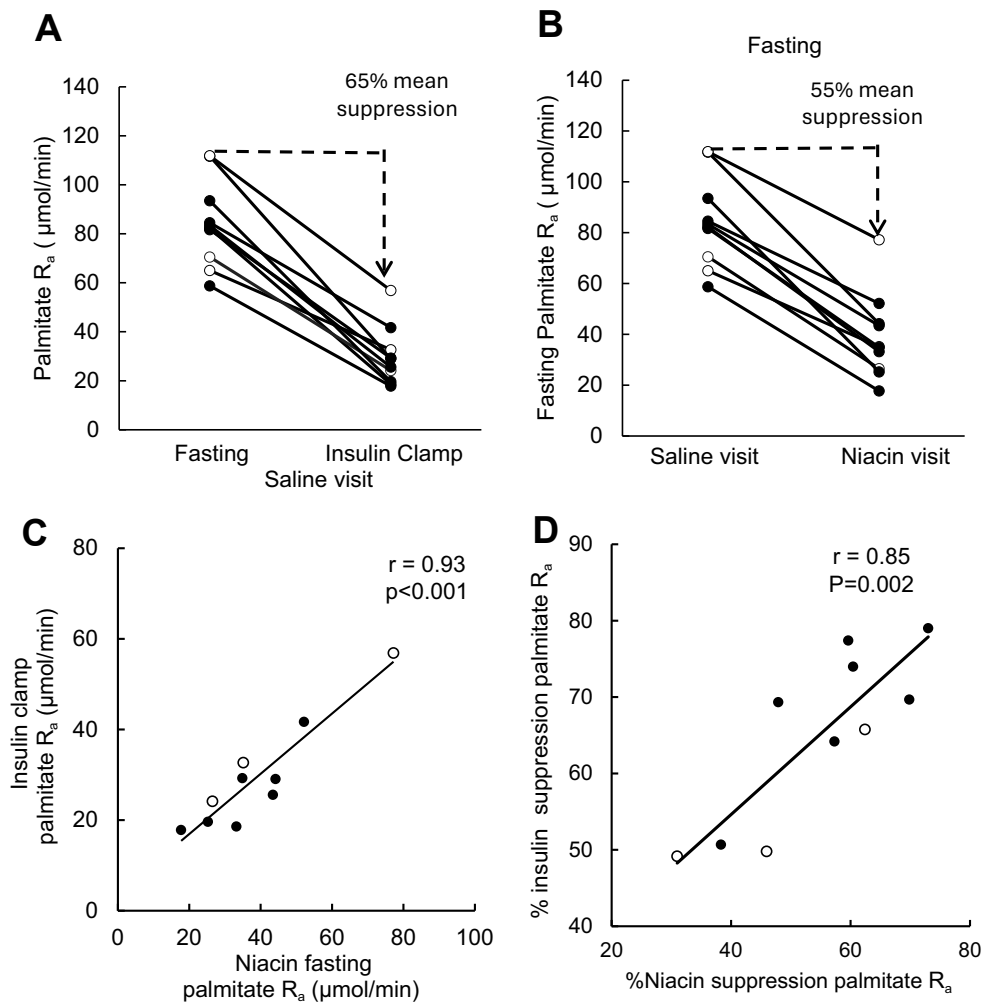


Figure 3—A: Fasting plasma palmitate R_a before and during insulin clamp on saline control visit. T2DM (\circ), UBO (\bullet). B: Fasting plasma palmitate R_a on saline control and niacin visits. C: Plasma palmitate R_a under fasting conditions on the niacin infusion day (x-axis) and during the insulin clamp on the saline day (y-axis). D: Percentage suppression of fasting palmitate R_a on niacin day (x-axis) and during insulin clamp on saline day (y-axis). Two-tailed P value calculated by Pearson correlation. $P < 0.01$ indicates statistical significance.

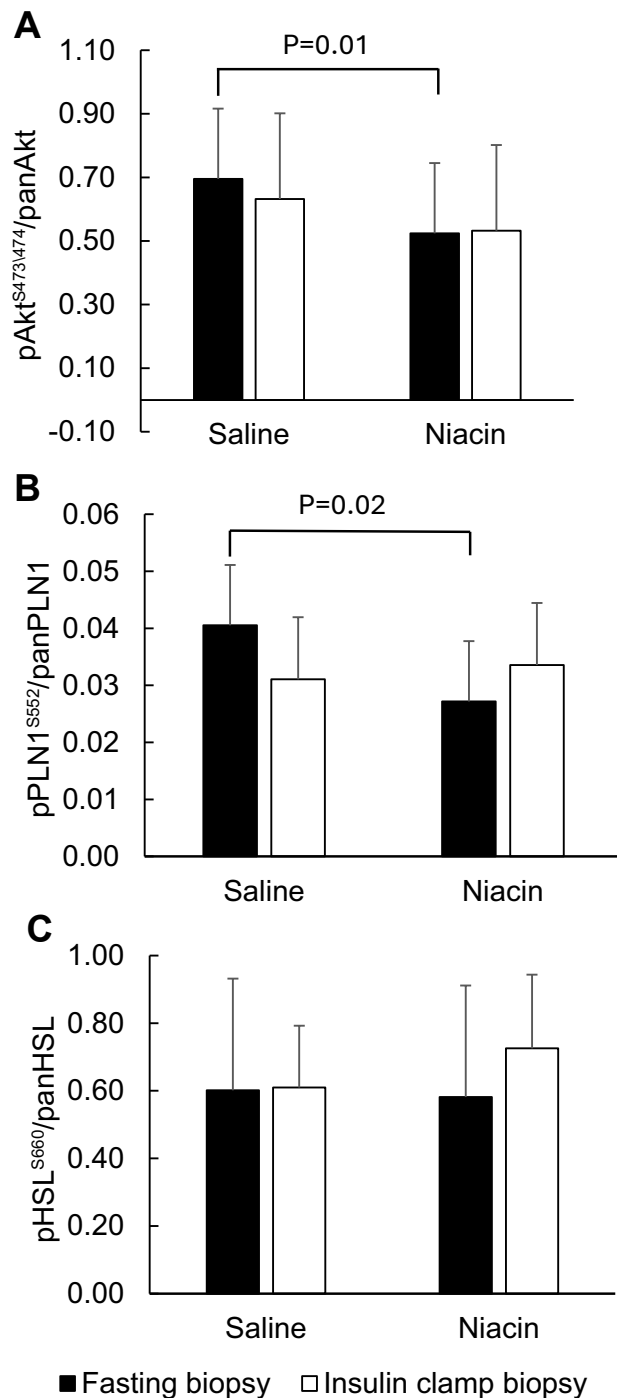


Figure 4—A: Ratio of pAkt^{S473/474} to panAkt on saline and niacin visit ($n = 9$). B: Ratio of pPLN1^{S552} to panPLN1 on saline and niacin visit ($n = 10$). C: Ratio of pHSL^{S660} to panHSL on saline and niacin visit ($n = 10$). Data are expressed as mean \pm SD. Two-tailed P value by paired sample t test.

measure systemic AT responsiveness to suppressive stimuli. As expected, the intravenous niacin suppressed postabsorptive FFA concentrations by inhibiting AT FFA release. Of great interest, the ability of niacin to suppress lipolysis was strongly correlated with the ability of insulin to suppress lipolysis (Fig. 3). Thus, the dysregulated suppression

of AT lipolysis in humans with obesity is not isolated to insulin.

Previous studies have attempted to elucidate the molecular underpinnings of AT insulin resistance in obesity using ex vivo human AT or animal models. Some investigators reported defects in signaling via the canonical, proximal insulin pathways (14–17), whereas others noted that these proximal defects affect glucose uptake in AT, but not suppression of lipolysis (18,19). If the reduced ability of insulin to suppress lipolysis in obesity is due specifically to alterations in signaling at or proximal to Akt, we predicted that the in vivo response to niacin, which suppresses lipolysis through a different proximal pathway (6), would be unrelated to the insulin responsiveness of AT lipolysis. Instead, we find that, for humans with obesity and/or T2DM, those whose AT were less responsive to the antilipolytic actions of insulin were also less responsive to the antilipolytic actions of niacin. In addition, the relationship between the suppression achieved by these two antilipolytic agents is strikingly strong (Fig. 3). These findings strongly suggest that what we refer to as “AT insulin resistance,” is not specific to insulin and that it involves pathways at or distal to where the actions of insulin and niacin converge to inhibit lipolysis, a site that has not been identified. The concepts behind these findings are depicted in Fig. 5. We propose that instead of a specific adipocyte “insulin resistance,” that the defects in lipolysis suppression in humans with obesity and/or T2DM involve more distal lipolysis-regulation pathways that are not specific to insulin.

In an attempt to interrogate the AT responses to niacin and insulin, we examined the phosphorylation states of one protein (Akt) that is in the proximal part of the insulin signaling pathway (6,18,20–23) and two proteins that are distal regulators of lipolysis (HSL and PLN1). We found that fasting Akt phosphorylation at S473/474 was less after an overnight niacin infusion than after the overnight saline infusion. A similar finding was reported by investigators who infused intravenous niacin into rats and found reduced AT Akt phosphorylation (6). While the exact mechanism remains unknown, one in vitro study suggests niacin may decrease Akt phosphorylation in human adipose-derived stem cells through increased expression of miRNAs, but more studies are needed to confirm this finding in humans in vivo (24). Unexpectedly, however, we did not observe an increase in Akt phosphorylation in response to insulin infusion on either study day, despite a significant insulin-mediated suppression of lipolysis. This observation contrasts with reports of increased Akt phosphorylation in adipocytes using cell models, animal models, and bolus insulin injections in lean adult humans (18–23). Possible explanations for the discrepant findings are that the AT Akt phosphorylation response to an insulin bolus (23) is different from constant insulin infusion, or that the elevated fasting plasma insulin concentrations in our volunteers with obesity resulted in generous Akt phosphorylation at S473/474, such

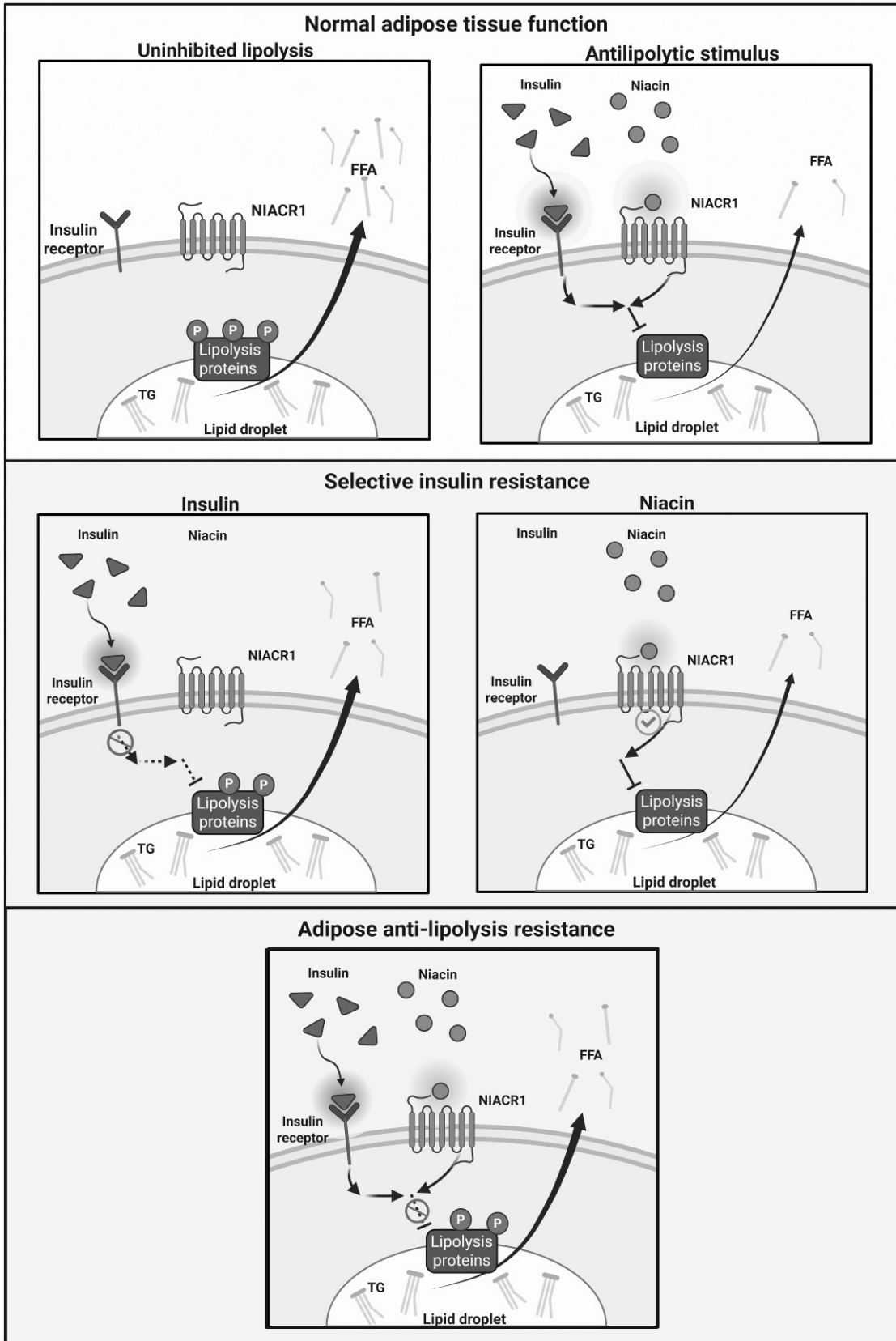


Figure 5—Concept figure of normal AT lipolysis, selective lipolysis insulin resistance, and broad adipose antilipolysis resistance. Normal AT function: Under uninhibited conditions (top left panel), AT lipolysis is active under conditions where phosphorylated lipolysis proteins hydrolyze triglycerides (TG) to FFA, which are released into the circulation. Under the influence of the antilipolytic stimuli (top right panel) insulin or niacin, lipolysis proteins are dephosphorylated, suppressing TG hydrolysis and thus FFA release. Insulin and niacin signal

that we could not detect further increases in Akt phosphorylation at this site in response to an insulin clamp. Nonetheless, the fact we observed considerable suppression of lipolysis without an increase in phosphorylation suggests that Akt, at least at the S473/474 site, is not the defining regulator of insulin-mediated lipolysis suppression in humans. We note that recent findings using cell lines and animal models suggest that insulin may suppress lipolysis through an Akt-independent pathway (18,19,25). While we cannot exclude the role of Akt phosphorylation (especially on other phosphorylation sites) in insulin suppression of lipolysis, it does not appear to be the culprit behind impaired antilipolysis response to insulin and niacin in human obesity. We therefore examined the response of more distal lipolysis protein (HSL and PLN1) to insulin and niacin.

To our knowledge, this is the first report of how the lipolysis proteins HSL and PLN1 phosphorylation respond to niacin and insulin in humans, *in vivo*. We found that the overnight niacin infusion was associated with a decrease in basal pPLN1^{S533} compared with the saline control study day. This is consistent with the concept that PLN1 phosphorylation plays a role in the regulation of lipolysis in human adipocytes and suggests that niacin action involves PLN1. We did not observe lesser, baseline HSL phosphorylation at the S660 site on the niacin compared with the saline control day, despite the significant suppression of lipolysis in response to niacin. This implies to us that niacin's inhibitory effect on lipolysis is not mediated by HSL at the S660 site. Finally, we did not detect significant changes in PLN1 or HSL phosphorylation in response to the insulin clamp. Consistent with our results, Hansen et al. (26) observed no change in phosphorylation of HSL at the S563 site or PLIN at the S522 site after insulin treatment in isolated human adipocytes. Instead, insulin caused microsegmentation of HSL and PLIN proteins, which may be a mechanism to reduce lipolysis activity (26). Nonetheless, we cannot exclude the possibility that insulin regulates lipolysis through dephosphorylation of HSL and PLIN1 at sites other than those we assayed; previous studies reported HSL and PLIN1 phosphorylation on multiple sites in addition to the ones we measured (26–31).

Our study has several strengths. By administering niacin intravenously, we could create stable plasma concentrations that are reported to result in maximal, achievable suppression of adipocyte lipolysis (12). This strategy complements the choice of our insulin infusion rate during the insulin clamp, which also creates insulin concentrations that result in near maximal, achievable lipolysis suppression in humans (13). Another strength is the use of isotopic FFA

tracers to quantitate the effects of both insulin and niacin on FFA release rates under conditions of overnight fasting and niacin-suppressed and insulin-suppressed conditions. Every participant serves as their own control in this study, thus allowing for paired comparisons of insulin versus niacin action on AT FFA release. Finally, our study recruited volunteers with obesity and/or T2DM and achieved a threefold range of AT insulin responses, which, given the reproducibility of our measures (13), allowed us to detect the strong relationship between insulin-mediated and niacin-mediated suppression of FFA-palmitate release rates.

Our study is not without limitations. Because of the intense nature of the study, we have a relatively small sample size. From the perspective of statistical power, however, this limitation did not prevent us from detecting the strong relationship between insulin and niacin effects on AT lipolysis. The relatively small sample size may have limited our ability to detect differences in adipocyte protein phosphorylation responses. Next, we observed large interindividual variability in the plasma concentrations of niacin and its metabolites. This is likely explained by the known differences in people's capacity to metabolize niacin (32,33). Nonetheless, the plasma niacin concentrations we achieved (200–800 ng/mL) are above those needed to induce the maximal, achievable suppression of lipolysis in human adipocytes (~100 ng/mL) (12). Lastly, we were only able to measure three AT proteins and one phosphorylation site on each protein due to the limited AT sample collected and the limited availability of antibodies that can be used to detect relevant human AT proteins. Although we tested several antibodies for human AT proteins (and their phosphorylation sites) in the lipolysis pathway, the ones we selected were based on specificity and responsiveness. If there were satisfactory antibodies and adequate amounts of tissue available, our study could be strengthened by examining multiple phosphorylation sites and other proteins in the lipolysis regulation pathway. Future studies are needed to elucidate the mechanisms behind resistance to insulin and niacin suppression of AT lipolysis in human.

Conclusion

In conclusion, we found a strong correlation between responsiveness to niacin and insulin with regards to the suppression of lipolysis in adults with obesity and/or T2DM. Given that niacin and insulin work through different proximal pathways, our finding indicates dysregulated adipocyte lipolysis may be due to defects in the more distal lipolysis pathway steps. Thus, instead of understanding

through different proximal pathways. Selective insulin resistance: Failure to normally suppress FFA release in obesity (middle left panel) results from specific defects in the proximal insulin signaling pathway, resulting in impaired insulin-induced suppression of lipolysis, while retaining normal niacin-induced suppression of lipolysis (middle right panel). We found that the lipolysis suppression responses to insulin and niacin to suppress lipolysis were highly correlated in human obesity. This indicates defects in the distal lipolysis pathway in obesity, suggesting a broad resistance to antilipolytic stimuli (bottom panel) and perhaps a more global AT antilipolysis resistance. NIACR1, niacin receptor 1. Figure was created in BioRender, <https://BioRender.com/tggbji9>.

adipose lipolysis in terms of specific “insulin resistance,” we suggest that there is a more global impairment in lipolysis suppression of subcutaneous AT of humans with obesity. Future studies should be directed toward understanding whether the defects in control of AT lipolysis are specifically in the realm of suppression or whether there is a generalized inflexibility in lipolysis regulation.

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