Subcutaneous Adipose Tissue Insulin Resistance Is Associated with Visceral Adiposity in Postmenopausal Women

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Objective: Whole body and subcutaneous adipose tissue (SAT) insulin resistance association with regional fat mass (FM) was determined.

Methods: Postmenopausal women (mean ± SD; age 56 ± 4 years, n = 25) who were overweight or obese (BMI 29.9 ± 5.1 kg/m²) were studied. Whole body and regional FM were measured by dual-energy X-ray absorptiometry (DXA) and computed tomography (CT). Women were studied during basal and insulin-stimulated (3-stage euglycemic clamp) conditions. Whole-body lipolysis was assessed by [²H₅]-glycerol rate of appearance and abdominal and femoral SAT lipolysis by interstitial glycerol (microdialysis).

Results: Whole body insulin resistance in skeletal muscle (insulin-stimulated glucose disposal) and adipose tissue (insulin-suppressed lipolysis) were independently related to trunk FM (r = −0.336 and 0.484, respectively), but not leg FM (r = −0.142 and −0.148, respectively). Local antilipolytic insulin resistance in abdominal, but not femoral, SAT was positively related to trunk FM (r = 0.551) but not related to leg FM (r = −0.289). Whole body and abdominal, but not femoral, adipose tissue insulin sensitivity were strongly related to skeletal muscle insulin sensitivity (r = −0.727 and −0.674, respectively).

Conclusions: The association of SAT insulin sensitivity (lipolysis) with adiposity and skeletal muscle insulin sensitivity was specific to the abdominal region.

Introduction

In normal weight individuals, the basal and postprandial (insulin-suppressed) release of nonesterified fatty acids (NEFA) during lipolysis of adipose tissue triglycerides is well-regulated to meet energy demands (1). In contrast, in obese individuals basal NEFA concentrations are elevated and insulin suppression of lipolysis is impaired, suggesting dysregulation with obesity (2-5). Although increased NEFA in obesity was generally thought to be proportional to fat mass, a review suggested that fasting NEFA (i.e., basal lipolysis) does not go up in proportion to total fat mass and may, in fact, be reduced per kilogram of fat with increasing obesity (at least in men) (6). Associations between reduced abdominal subcutaneous adipose tissue (SAT) basal lipolysis and increased fasting hyperinsulinemia suggests there may be a progressive downregulation of lipolysis with increasing systemic glucoregulatory insulin resistance (6). The ability of insulin to effectively suppress lipolysis is important because most individuals spend the majority of the day in a postprandial rather than fasted state. Yet, it is not known whether systemic insulin-suppressed lipolysis, like basal lipolysis, is also down-regulated with increasing adiposity and glucoregulatory insulin resistance. Moreover, it remains unclear whether local (adipose tissue-specific) insulin-suppressed lipolysis is proportional to local fat mass or indicative of impaired SAT function. In vitro data from subcutaneous (abdominal and gluteal) adipocytes of obese premenopausal women demonstrated a correlation of insulin resistance with visceral adiposity (7). Local resistance to insulin may provide insights into SAT dysfunction and redistribution away from subcutaneous and toward visceral depots with increasing obesity and may be particularly important after menopause when women begin to accumulate more visceral fat (8). Indeed, in vitro data suggested that the higher adipocyte insulin sensitivity in gluteal, compared to abdominal, that was present in obese premenopausal women (7) was...
Multiple Institutional Review Board. Participate in the study, which was approved by the Colorado fpsystem function. All participants provided written informed consent to postmenopausal women. Be particularly apparent in a cohort of overweight and obese SAT lipolysis with hyperinsulinemia or visceral adiposity. We expected that any associations of abdominal and femoral SAT). We expected that any associations of SAT lipolysis with hyperinsulinemia or visceral adiposity would be particularly apparent in a cohort of overweight and obese postmenopausal women.

Methods

Subjects

We retrospectively analyzed baseline data collected in healthy, sedentary postmenopausal women (n = 25) previously enrolled in two studies conducted by our laboratory. Some of the data have been reported previously (10-12). Postmenopausal status was defined as cessation of menses for at least 1 year or hysterectomy with a FSH > 30 IU/L. Women were excluded if they were currently using hormone therapy, had a history of hormone-sensitive cancer, fasted plasma glucose > 5.6 mmol/l, uncontrolled hypertension (resting systolic blood pressure > 150 mmHg or diastolic > 90 mmHg), thyroid dysfunction (TSH < 0.5 or > 5.0 mU/ml), hypertriglyceridemia (fasting triglycerides > 4.5 mmol/l), or abnormal liver or renal function. All participants provided written informed consent to participate in the study, which was approved by the Colorado Multiple Institutional Review Board.

Body composition

Total fat mass (FM) and fat-free mass (FFM) were measured by dual-energy X-ray absorptiometry (DXA) using Lunar DPX-IQ (n = 15; Software v4.38, Lunar Co., Madison, WI) or Hologic Delphi-W (n = 14; software v11.2, Hologic, Bedford, MA). The recommendations of the manufacturers were used to define the trunk and leg regions. As previously reported (13), the use of two DXA instruments could not be avoided, so orthogonal regression equations were generated from a separate cohort of subjects (n = 48) measured on both instruments to adjust Lunar data to Hologic. The average between-instrument biases for Hologic vs. Lunar were: 0.17 kg body mass, −0.75 kg total FM, −0.93 kg trunk FM, −0.34 kg leg FM, and 0.92 kg FFM.

Abdominal (visceral and subcutaneous) and mid thigh (subcutaneous) fat areas were determined by computed tomography (CT) as previously described (14). Single slice images were obtained at the levels of the L2-L3 and the L4-L5 intervertebral spaces and the mid-thigh. The abdominal visceral fat areas (cm²) were manually defined by tracing the muscles of the abdominal wall. Abdominal subcutaneous fat areas (cm²) were calculated by subtracting the visceral fat areas from the total abdominal fat area. CT slice fat areas were converted to mass and used to estimate total upper body visceral and subcutaneous fat mass as previously described (15). In brief, fat areas were averaged over the two abdominal slices, multiplied by the slice thickness (10 cm) to calculate fat volume (cm³), and converted to mass (0.9 kg triglyceride/l tissue). Visceral and subcutaneous FM (kg) in the CT abdominal slices were then multiplied by the DXA trunk FM to estimate the proportions of total visceral and subcutaneous fat in the upper body.

Hyperinsulinemic, euglycemic clamp

Three-stage (4, 8, and 40 mU/m²/min) hyperinsulinemic, euglycemic clamps were administered as previously described (10). Briefly, clamps were performed on the Clinical and Translational Research Center (CTRC) following a, 3-day standardized diet and 12-h fast. Plasma glucose was obtained at bedside every 5 min and the dextrose infusion was adjusted to sustain plasma glucose at 5 mmol/l. A primed (1.5 μmol/kg), constant (~0.1 μmol/kg/min) infusion of [1H3]glycerol (Cambridge Isotope Laboratories, Andover, MA) was delivered throughout a 90-min basal period and the three 90-min insulin stages to measure whole-body lipolysis. Blood samples were collected before (fasted) the start of infusions and at 60, 75, and 90 min of the basal period and each insulin stage for determination of insulin, and glycerol (concentration and isotope enrichment). Steady-state insulin and whole body glucose disposal rate (GDR; mg/kg/min) were determined from the average insulin concentration and steady-state glucose infusion rate, respectively, during the final 30 min of the 40 μM/min dose insulin infusion.

Microdialysis

Regional SAT lipolysis was evaluated by placing linear microdialysis probes (BAS, custom LM-3 probes, 3 cm membrane) in both abdominal and femoral SAT as previously described (12). Briefly, the microdialysis probes were inserted under sterile technique into abdominal (2 probes lateral to the umbilicus; ~3 cm apart) and femoral (2 probes mid-thigh; ~3 cm apart) SAT. Throughout the insulin clamp procedure the probes were perfused at 2.0 μl/min with Ringer solution containing 2.5 mM glucose, 200 μM [13C] glycerol, and 5 mM ethanol. The outgoing dialysate was collected in 15-min fractions (30 μl) throughout the basal period and each insulin stage.

Whole-body lipolysis

Plasma glycerol concentrations were measured by the CTRC core laboratory. The analysis of [1H3] glycerol was performed by the Colorado Nutrition and Obesity Research Center Mass Spectrometry Core Laboratory using an adaptation of the negative ion chemical ionization gas chromatography–mass spectrometry as previously described (16). The average rate of appearance of glycerol (GLYRA) over the last 30 min of each stage was calculated using the steady-state equation of Steele (17): GLYRA = F/EL, where F is the rate of infusion of [1H3] glycerol (0.10 μmol/kg/min) and E is the plateau plasma isotope enrichment.

Regional SAT lipolysis

Dialysate samples were batched and sent to a commercial mass spectrometry laboratory (Metabolic Solutions) for the analysis of [13C3] glycerol enrichment and to East Carolina University for the spectrophotometric measurement of glycerol concentrations using an automated CMA600 Microdialysate Analyzer (CMA Microdialysis, Acton, MA). [13C3] glycerol isotopic tracer was included in the
Table 1: Subject characteristics

| Variable                | Mean ± SD | Variable                | Mean ± SD |
|-------------------------|-----------|-------------------------|-----------|
| Age (years)             | 56 ± 4    | Abdominal subcutaneous FM (kg) | 12.1 ± 4.1 |
| Years since menopause   | 10 ± 7    | Abdominal visceral FM (kg)  | 4.4 ± 2.1  |
| Weight (kg)             | 77.7 ± 14.6 | Fasted glucose (mmol/l)     | 4.8 ± 0.4 |
| BMI (kg/m²)             | 29.3 ± 5.0  | Whole body GDR (mg/kg/min)| 5.6 ± 2.8 |
| Total FM (kg)           | 33.0 ± 8.3 | Whole body EC₅₀ (pmol/l)    | 88.3 ± 39.5|
| Trunk FM (kg)           | 16.6 ± 5.7 | Abdominal SAT EC₅₀ (pmol/l)| 99.5 ± 42.0|
| Leg FM (kg)             | 11.8 ± 3.5 | Femoral SAT EC₅₀ (pmol/l)  | 102.9 ± 35.5|
| Fat-free mass (kg)      | 44.2 ± 5.8 |                         |           |

EC₅₀ = insulin concentration needed to half-maximally suppress lipolysis; FM = fat mass; GDR = insulin-mediated glucose disposal rate; SAT = subcutaneous adipose tissue.

Data analysis
Exponential decay curves for glycerol (whole body GLYRA, SAT intersitial concentration) across the range of insulin concentrations were generated for each individual and suppression of lipolysis was calculated as the insulin concentration needed to half-maximally suppress glycerol (EC₅₀) as previously described (10). The EC₅₀ was calculated for the whole body, abdominal SAT, and femoral SAT. Pearson’s correlations were used to evaluate the associations among measures of whole body and regional antilipolytic insulin action (EC₅₀), whole body glucoregulatory insulin action (GDR), and adiposity (subcutaneous and visceral fat mass). Partial correlations were used to test whether the associations of upper body (trunk fat) and lower body (leg fat) adiposity with measures of insulin sensitivity remained or changed after controlling for the other region. All statistical analyses were performed using SPSS software (IBM SPSS Statistics 21.0).

Results
Study subjects were postmenopausal women who were sedentary and overweight or mildly obese but otherwise healthy (Table 1). None of the subjects had diabetes, smoked, or used hormone therapy or lipid- or glucose-lowering medications at the time of the study. As expected, whole body and regional SAT insulin resistance were directly correlated with fasted insulin concentration (Figure 1a). Whole body and abdominal, but not femoral, SAT insulin-suppression of lipolysis were also inversely related to whole body skeletal muscle insulin-stimulated glucose disposal (Figure 1b). In addition, systemic glucoregulatory (GDR), and antilipolytic (ED₅₀) insulin action were directly correlated with all measures of adiposity (Table 2). Local abdominal SAT insulin resistance was correlated with upper but not lower body adiposity, whereas femoral SAT insulin resistance was not correlated with any measure of adiposity (Table 2). Trunk fat mass remained significantly correlated with measures of systemic and abdominal SAT insulin sensitivity after controlling for leg fat mass. However, there was no independent relation between leg fat mass and insulin sensitivity after controlling for trunk fat mass (Table 2).

Discussion
The primary new finding of the current study was that systemic and local SAT insulin resistance (measured in vivo) was directly correlated with abdominal (subcutaneous and visceral), but not femoral, fat mass in postmenopausal women. Whole body and abdominal, but not femoral, SAT insulin resistance were also inversely related to skeletal muscle insulin resistance. The independent association of upper body (trunk) fat with whole body and abdominal SAT insulin resistance persisted after controlling for lower body (leg) fat. In contrast, after controlling for upper body fat, the independent association of lower body fat with insulin resistance disappeared or was reversed (i.e., appeared protective).

Our data are consistent with previous studies comparing abdominal and gluteal adipocyte insulin sensitivity in vitro, but extend previous studies by measuring whole body and regional adipose tissue insulin sensitivity in vivo using microdialysis and a 3-stage hyperinsulimemic, euglycemic clamp in postmenopausal women. Previous studies in obese premenopausal women showed that abdominal adipocytes were less sensitive to the antilipolytic action of insulin than gluteal adipocytes yet insulin sensitivity in adipocytes from both regions was associated with visceral adiposity (7). Likewise, systemic antilipolytic insulin resistance was correlated with visceral adiposity in obese premenopausal women (19). Similar to previous studies we found systemic antilipolytic insulin resistance was correlated with visceral adiposity, but in contrast we found that only abdominal, not femoral, SAT insulin sensitivity was associated with visceral adiposity. Differences between studies may be attributable to the fact that our cohort was postmenopausal, our lipolysis measurements were done in vivo, or we studied femoral rather than gluteal tissue. However, our in vivo data and previous in vitro data (9) did not detect region-specific differences in SAT insulin sensitivity among postmenopausal women, so reduced insulin sensitivity in lower body fat does not appear to explain the lack of association with visceral...
Our current study was also consistent with a previous study which showed a strong correlation between adipocyte insulin sensitivity in vitro and systemic fasted hyperinsulinemia among postmenopausal women (9). We extended this observation by demonstrating a strong inverse association between abdominal SAT insulin resistance and skeletal muscle insulin resistance. Our results also extend the observations made in men that abdominal SAT basal lipolysis is directly associated with increased fasted hyperinsulinemia (6). Thus, this study of postmenopausal women in vivo is consistent with and extends previous studies. Together the data suggest there may be a progressive downregulation of the ability of insulin to suppress lipolysis in the abdominal SAT depot with increasing upper body adiposity and systemic glucoregulatory insulin resistance.

Numerous studies have demonstrated elevated basal and postprandial NEFA in obese compared to lean individuals (2-5). The elevated NEFA in obesity was previously thought to be proportional to total adiposity, but a more recent review of the literature suggested basal lipolysis is not proportionally increased, and may actually be decreased, with increasing fat mass (at least in men) (6). Whether this is also true under insulin-stimulated conditions was not known, but 24-h studies (much of which is spent in the postprandial state) suggested there may be less NEFA released per unit of fat tissue in abdominally obese, compared to lean, men (20). In support of this, our data in overweight and postmenopausal women suggest that resistance to the antilipolytic action of insulin increases directly with abdominal adiposity, resulting in disproportionately low release of NEFA. Such attenuation in adipose tissue NEFA release in abdominal obesity might be a functional adaptation that minimizes lipid mobilization in the face of increased fat availability. However, it remains unclear why such a functional adaptation would not also occur in femoral tissue.

The association between adipose tissue insulin resistance and fat mass in our study was unique to abdominal tissue; femoral adipose tissue insulin resistance was not related to fat mass. Although leg fat mass was associated with systemic glucoregulatory and antilipolytic insulin resistance, these correlations were no longer significant after adjusting for trunk fat mass. This is consistent with a recent study in middle-aged and older adults which showed a favorable association of thigh fat with systemic insulin sensitivity after accounting for

### Table 2

|                     | Whole body GDR | Whole body EC<sub>50</sub> | Abdominal SAT EC<sub>50</sub> | Femoral SAT EC<sub>50</sub> |
|---------------------|----------------|---------------------------|-------------------------------|------------------------------|
| Pearson correlations|                |                           |                               |                               |
| Whole body FM       | −0.673*        | 0.590*                    | 0.516*                        | 0.141                        |
| Trunk FM            | −0.651*        | 0.609*                    | 0.557*                        | 0.134                        |
| Leg FM              | −0.603*        | 0.444*                    | 0.302                         | 0.128                        |
| Abdominal subcutaneous FM | −0.565*      | 0.464*                    | 0.538*                        | 0.087                        |
| Abdominal visceral FM | −0.687*     | 0.770*                    | 0.511*                        | 0.195                        |
| Partial correlations|                |                           |                               |                               |
| Trunk FM (adj. for leg FM) | −0.336†      | 0.484*                    | 0.552*                        | 0.048                        |
| Leg FM (adj. for trunk FM) | −0.142        | −0.148                    | −0.289                         | 0.028                        |

*P < 0.05; †P = 0.11; EC<sub>50</sub> = insulin concentration needed to half-maximally suppress lipolysis; FM = fat mass (kg); GDR = insulin-mediated glucose disposal rate (mg/kg/min); SAT = subcutaneous adipose tissue.
differences in visceral adiposity (21). These findings were not surprising given that femoral fat mass generally appears benign with respect to disease risk even in the context of overweight and obesity (14,22). Moreover, upper body subcutaneous adipose tissue is thought to be the main site of storage and release of fatty acids (23). Deposit-specific differences may be due to differences in storage (re-esterification of fatty acids as triglyceride) instead of differences in NEFA release because antilipolytic insulin sensitivity (EC50) was similar between abdominal and femoral adipose tissue depots (99.5 ± 42.0 vs. 102.9 ± 35.5 pmol/L, respectively). Of note, as a percentage of lipolysis, basal whole body re-esterification rates are three-fold higher in postmenopausal women compared to premenopausal women (24), but whether there are depot-specific differences in re-esterification among postmenopausal women to our knowledge remains unknown.

We acknowledge that the observed correlations do not infer causality. Studies are needed to determine whether improvement in abdominal SAT insulin sensitivity favorably reduces visceral fat accumulation and improves glucoregulatory insulin sensitivity. Evidence for this comes from studies of thiazolidinediones (TZDs) used to treat patients with type 2 diabetes. TZDs are peroxisome-proliferator activated receptor γ (PPARγ) agonists known to increase adipose tissue insulin sensitivity (suppression of lipolysis) (25). In addition to improving suppression of lipolysis, PPARγ is a master transcription regulator of adipogenesis, so it is not surprising that TZDs are known to increase subcutaneous fat (26). Importantly, these increases in subcutaneous fat are not accompanied by increases, but rather slight decreases, in visceral fat and improvements in skeletal muscle glucose uptake (26). Whether the reductions in visceral fat are causally related to changes in glucoregulatory insulin sensitivity remains unknown (27,28), but the TZD data support the hypothesis that improving subcutaneous insulin sensitivity and fat storage reduces visceral fat and improves glucoregulatory insulin sensitivity.

It is important to note that the generalizability of our results may be limited; we studied a small group of sedentary and overweight to moderately obese, but otherwise healthy, postmenopausal women. Whether these results apply to men or younger adults cannot be determined. Our results are strengthened by the fact that these were well-controlled physiologic studies that used the reference methods to assess both whole body (multistage hyperinsulinemic-euglycemic clamp with isotope tracers) and tissue-specific (adipose tissue microdialysis) insulin-mediated suppression of lipolysis. With increasing obesity, adipose tissue remains relatively much more sensitive to the antilipolytic action of insulin when compared with insulin-stimulated glucose uptake in skeletal muscle. Nevertheless, our data suggest that the degree of adipose tissue insulin resistance varies markedly among overweight and obese postmenopausal women and is proportional to skeletal muscle insulin resistance and hyperinsulinemia.

In conclusion, abdominal, but not femoral, subcutaneous adipose tissue antilipolytic insulin resistance was related to abdominal (subcutaneous and visceral) fat mass and skeletal muscle glucoregulatory insulin resistance. We postulate that abdominal adipose tissue insulin resistance is a marker of adipose tissue dysfunction and may drive abdominal fat accumulation and skeletal muscle insulin resistance. Future studies are needed to determine whether improving subcutaneous adipose tissue antilipolytic insulin sensitivity reduces abdominal fat accumulation and skeletal muscle insulin resistance and to investigate the mechanisms by which this occurs.  

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