IL-15 concentrations in skeletal muscle and subcutaneous adipose tissue in lean and obese humans: local effects of IL-15 on adipose tissue lipolysis

Joseph R. Pierce,¹² Jill M. Maples,¹² and Robert C. Hickner¹⁻⁵

¹Human Performance Laboratory, East Carolina University, Greenville, North Carolina; Departments of ²Kinesiology and ³Physiology and ⁴Center for Health Disparities, East Carolina University, Greenville, North Carolina; and ⁵Discipline of Biokinetics, Exercise, and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Submitted 8 December 2014; accepted in final form 27 April 2015

Pierce JR, Maples JM, Hickner RC. IL-15 concentrations in skeletal muscle and subcutaneous adipose tissue in lean and obese humans: local effects of IL-15 on adipose tissue lipolysis. Am J Physiol Endocrinol Metab 308: E1131–E1139, 2015. First published April 28, 2015; doi:10.1152/ajpendo.00575.2014.—Animal/cell investigations indicate that there is a decreased adipose tissue mass resulting from skeletal muscle (SkM) IL-15 secretion (e.g., SkM-blood-adipose tissue axis). IL-15 could regulate fat mass accumulation in obesity via lipolysis, although this has not been investigated in humans. Therefore, the purpose was to examine whether SkM and/or subcutaneous adipose tissue (SCAT) IL-15 concentrations were correlated with SCAT lipolysis in lean and obese humans and determine whether IL-15 perfusion could induce lipolysis in human SCAT. Local SkM and abdominal SCAT IL-15 (microdialysis) and circulating IL-15 (blood) were sampled in lean (BMI: 23.1 ± 0.9 kg/m²; n = 10) and obese (BMI: 34.7 ± 3.5 kg/m²; n = 10) subjects at rest/during 1-h cycling exercise. Lipolysis (SCAT interstitial glycerol concentration) was compared against local/systemic IL-15. An additional probe in SCAT was perfused with IL-15 to assess direct lipolytic responses. SkM IL-15 was not different between lean and obese subjects (P = 0.45), whereas SCAT IL-15 was higher in obese vs. lean subjects (P = 0.02) and was correlated with SCAT lipolysis (r = 0.45, P = 0.05). Exercise increased SCAT lipolysis in lean and obese (P < 0.01), but exercise-induced SCAT lipolysis changes were not correlated with exercise-induced SCAT IL-15 changes. Microdialysis perfusion resulting in physiological IL-15 concentrations in the adipose tissue interstitium increased lipolysis in lean (P = 0.04) but suppressed lipolysis in obese (P < 0.01). Although we found no support for a human IL-15 SkM-blood-adipose tissue axis, IL-15 may be produced in/act on the abdominal SCAT depot. The extent to which this autocrine/paracrine IL-15 action regulates human body composition remains unknown.

Address for reprint requests and other correspondence: R. C. Hickner, Human Performance Laboratory, 363 Ward Sports Medicine Bldg., Greenville, NC 27858 (e-mail: Hickner@ecu.edu).

http://www.ajpendo.org 0193-1849/15 Copyright © 2015 the American Physiological Society E1131


mcdialysis; myokine; adipokine; interleukin; adipose tissue metabolism

THE COMMUNICATION OF CYTOKINES within and between organ systems such as skeletal muscle (SkM) and adipose tissue is potentially involved in human obesity, given the antiobesogenic effects of some cytokines reported in studies using animal and cell models. Interleukin (IL-15), with a particularly high SkM expression and relatively low adipose tissue expression (13, 29), is one cytokine demonstrated to have additional nonimmune metabolic roles. For example, exogenous IL-15 delivery has been reported to decrease white adipose tissue (WAT) mass 14–44% in several animal strains (3, 7). Moreover, compared with wild-type counterparts and independent of food intake, IL-15 genetic knockout (KO) animals gained body weight and fat mass, whereas IL-15 overexpression animals demonstrated lower body mass and fat mass (5, 6).

The strongest evidence for IL-15’s potential role in the regulation of obesity was provided by altering IL-15 expression in muscle and by altering the amount of IL-15 released into the circulation, generating a SkM-blood-adipose tissue gradient for IL-15 (28). Quinn et al. (28) demonstrated that overexpression of intracellular SkM IL-15 did not produce any changes in body composition, whereas overexpression of SkM IL-15 that was oversecreted into the circulation decreased fat mass significantly in male and female mice. Human studies indicate that 1) obese individuals have lower circulating IL-15 than their lean counterparts (6, 21) and 2) circulating IL-15 is negatively correlated with trunk fat mass and percent body fat (21). From this limited evidence, it has been speculated that human SkM IL-15 secretion accounts for differences in plasma IL-15. Conversely, obese SkM IL-15 mRNA expression was not related to plasma IL-15 (21), indicating that SkM may not contribute substantially to systemic IL-15. Evidence that adipocytes express IL-15 (1, 29) and that obesity is considered a low-grade inflammatory condition (9) suggests that adipose tissue in obese humans may express/secrete more IL-15 than adipose tissue in lean individuals. To our knowledge, no studies have assessed human adipose tissue IL-15 concentrations, leaving the endocrine and autocrine/paracrine metabolic roles of IL-15 in human obesity unanswered.

Losses in fat mass associated with IL-15 upregulation/overexpression could result from decreased lipid uptake and incorporation as well as increased lipid mobilization, although supraphysiological IL-15 concentrations were used in the studies that describe these effects (2, 3, 6, 7). With specific regard to lipid mobilization, Ajuwon and Spurlock (2) demonstrated that porcine adipocytes exposed to IL-15 underwent increased lipolysis, and this response was blunted by blocking IL-15R cell signaling intermediates JAK and PKA. Although IL-15 modulates lipolysis in porcine cells, it remains to be determined whether IL-15 could induce lipolysis in human adipose tissue.

Despite attempts at identifying roles for IL-15 in obesity, sources of circulating IL-15 and/or stimuli leading to IL-15 secretion in humans remain unknown (6). Acute exercise increases lipolysis (4, 32) and could also be a potential stimulus for IL-15 secretion, yet both increases (8, 33) and no changes (22, 30) in IL-15 with exercise have been observed. Therefore, IL-15 could be partially responsible for exercise-induced lipolysis. In addition, IL-15 released from and acting within adipose tissue may represent an important autocrine/paracrine axis in which fatty acids are directed from the adipocyte toward other needs (2). It remains to be clarified...
whether IL-15 directly increases human adipose tissue lipolysis or whether this action is different between lean and obese humans, potentially playing a role in human obesity. Therefore, the purpose of this study was to determine the interstitial IL-15 concentrations in SkM and subcutaneous adipose tissue (SCAT) and to determine whether these concentrations are related to SCAT lipolysis in lean and obese humans at rest and during exercise. We also aimed to determine whether perfusion of IL-15 could directly induce in vivo lipolysis in human SCAT.

MATERIALS AND METHODS

Screening Visit

Participants. Potential subjects visited the Fitness Instruction, Training, & Testing building at East Carolina University (ECU), where they were briefed on all study procedures, and signed an informed consent form prior to any participation. Consented volunteers underwent a general health screening and completed a brief medical history questionnaire to confirm qualification. Ten lean (BMI: 23.1 ± 1.9 kg/m²; age: 24.0 ± 3.7 yr) and 10 obese (BMI: 34.7 ± 3.5 kg/m²; age: 27.3 ± 9.1 yr) subjects participated in this study. Demographic and body composition characteristics for study participants are provided in Table 1. Potential subjects were excluded if they did not fit within the desired BMI categories of lean (18–24.9 kg/m²) or obese (30–39.9 kg/m²), exercised 23.1 days/wk, were smokers, had any known cardiovascular or neuromuscular disease, were taking any medications affecting metabolism, or were pregnant or lactating. We did not control for the timing of the experimental visit within the menstrual cycle phase in female subjects. However, we did measure follicle stimulating hormone and luteinizing hormone and determined that there were no differences between the lean and obese females in either measurement (P = 0.59 and P = 0.42, respectively). All study procedures were approved by the ECU University and Medical Center Institutional Review Board.

Body composition assessment. Participants had height measured to the nearest 0.5 cm using a wall-mounted stadiometer, weight assessed on an electronic scale to the nearest 0.1 kg, and BMI (kg/m²) calculated. Detailed body composition analysis was measured with a dual-energy X-ray absorptiometry (DEXA) scan (Prodigy; GE Lunar, Madison, WI), where subjects lay on the DEXA table while a fan beam scanner obtained detailed lean and fat mass densitometries. All DEXA scans were performed by the same technician only after it was ensured that the instrument had passed a daily quality assurance protocol using a manufacturer-provided calibration block. Provided software calculated specific parameters, which included bone mineral density and content, fat mass and percent body fat (total and regional estimates), lean mass, and percent lean mass using standard anatomic landmarks.

Experimental Visit

Within 48 h of the experimental visit, subjects were instructed not to perform vigorous exercise or ingest alcohol. The day prior, they were asked to eat meals close to their typical diet and drink water only from ~2200 the night before. Subjects were asked to eat a provided breakfast 2 h before their experimental visit began (~0600), which consisted of two crunchy granola bars [190 kcal, 29 g of carbohydrate (CHO), 6 g of fat (FAT), 4 g of protein (PRO)] and 4 oz. of commercially available drink mix (125 kcal, 17 g of CHO, 4.5 g of FAT, 4.5 g of PRO) for a total of 315 kcal (~60% CHO/30% FAT/10% PRO). Subjects reported to the East Carolina Heart Institute for their experimental visit at ~0800. After it was confirmed that the meal was eaten at the time specified to standardize feeding, subjects were asked to rest in a semirecumbent supine position in a hospital bed to begin the microdialysis probe insertion for interstitial fluid/dialysate sampling and intravenous catheter insertion for blood draws. Other than the exercise portion, subjects were allowed to work quietly, read, or watch television throughout the visit. Subjects were allowed to drink water ad libitum during the day, but no food was allowed until the end of the experimental visit.

Microdialysis probes and perfusates. Custom microdialysis probes (CMA/20; 15- or 30-mm membrane length, 100-kDa cutoff) were ordered from CMA Microdialysis (CMA/Harvard Apparatus, Holliston, MA), soaked for 30 min in 70% isopropanol the day before use, and kept antiseptic in sterile water overnight. Perfusate solutions were prepared in a base solution of sterile 0.9% NaCl (saline), where we added 10 mM ethanol (EtOH) to estimate blood flow and calculate interstitial IL-15 and glycerol concentrations and 30 g/l Dextran (40 kDa molecular weight; Sigma Aldrich, St. Louis, MO) to prevent ultrafiltration across the large pore size membrane. For the IL-15 perfusion portion of the study, recombinant human IL-15 was purchased sterile filtered and lyophilized from PeproTech (Rocky Hill, NJ) and reconstituted as per the manufacturer’s instructions prior to use. IL-15 perfusate solutions of varying concentrations (0, 20, 650, 6,500, and 65,000 pg/ml) were prepared in a base solution of sterile 0.9% NaCl (saline) and 10 mM EtOH. We based our IL-15 perfusate concentrations on studies that measured circulating IL-15 concentrations in humans (6, 12, 15, 21), delivered exogenous IL-15 to animals (3, 6, 7), or incubated cells with IL-15 (2, 6). Importantly, there were no differences in measured perfusate concentrations between lean and obese subjects. Once agents were added and prepared to desired concentrations, perfusates were sterile filtered with 0.22-μm filters into CMA 106 microdialysis syringes (MDialysis, North Chelmsford, MA) and kept overnight at 4°C until use the next day.

Microdialysis probe insertion. For SkM and SCAT microdialysis probe insertion, the skin around the insertion site was first cleaned using iodine swab sticks. To ease any discomfort from the probe placement, a small amount (~1–2 ml/insertion site) of 1% lidocaine was then injected with the use of ethyl chloride spray (cold numbering
spray) under the skin surface and down into the vastus lateralis (VL) thigh muscle 5–6 cm proximal to the patella or in the abdominal SCAT space 2–3 cm lateral to the umbilicus. Next, a removable plastic sheet was inserted into the numbed area with the use of an 18-gauge catheter needle. Once the needle was removed, the sterile microdialysis probe was placed inside the plastic sheet. The plastic guide was then removed, leaving the dialysis membrane portion of the microdialysis probe embedded below the skin and into the VL muscle or SCAT.

Inserted microdialysis probes were secured to the subject and connected to perfusate syringes and calibrated syringe pumps (CMA 107 Pump; MDialysis). Once the pump was started with the flow rate set to 2 μl/min and flow was visually established, we allowed a 1-h perfusion (termed “equilibration period”) before initiating collection of dialysate for subsequent analysis. Dialysates were collected in 300-μl plastic microvials (Millipore, Columbus, OH) and kept at either 4 or 20°C until desired analysis occurred (see below). Dialysate vials were weighed both prior to and following collection to monitor flow rate.

Blood collection. Subjects had an intravenous catheter with a saline lock inserted into an antecubital vein in the forearm near the elbow. Line patency was maintained by periodically flushing with 2–3 ml of sterile saline. At the time indicated in Fig. 1, blood was drawn into vacutainers (BD Diagnostics, Franklin Lakes, NJ) containing EDTA for plasma or into serum separator tubes for serum. After seven to 10 inversions [serum clotted for 30 min at room temperature (RT)], vacutainers were spun for 15 min (plasma at 4°C, serum at RT), and plasma or serum was aliquotted to microcentrifuge tubes and frozen at −80°C until analysis.

Acute exercise protocol. To determine whether IL-15 expression/secretion was induced by exercise and/or related to exercise-induced lipolysis, subjects exercised on a Monark Ergomedic 828E cycle ergometer for 60 min (Monark Exercise). Participants were first provided a heart rate (HR) monitor (Polar Electro, Lake Success, NY) and obtained morning resting HR (HRrest) at home. The difference between measured HRrest and calculated HRmax (220 − age) was the HR reserve (HRR). On the day of the experimental visit, subjects were fitted with the same HR monitor and asked to pedal for 60 min at the training HR, using the Karvonen equation and 60% of the HRR: [(HRmax − HRrest) × 0.6] + HRrest. Exercise intensity was adjusted as necessary at the 55- to 60-rpm pedal rate to keep participants as close to their training HR as possible.

Basic blood biochemistry. Resting serum (8 h fasted from provided breakfast, end of stage 4; Fig. 1) was analyzed for basic blood chemistries using the automated Unicel Dxc 600i Synchron Access Clinical System (Beckman Coulter, Brea, CA), and the results are presented in Table 2. We measured glucose, insulin, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol. Using blood glucose and insulin, we calculated homeostatic model assessment (HOMA) to quantify insulin resistance using the equation HOMA = fasting glucose [mg/dl] × fasting insulin [μIU/ml]/405 (19).

Glycerol. For each microdialysis collection period (~30–45 min), indicated by “MD Sample” in Fig. 1, collected dialysate was assessed for glycerol on a specialized microdialysis analyzer (CMA 600; MDialysis) according to the manufacturer’s directions. The analyzer measures glycerol concentration from <2 μl samples using a colorimetric reaction. Dialysate samples were kept at −20°C until analysis. All samples were run in duplicate, measured against provided calibrators, and averaged for data analysis.

EtOH assay. During microdialysis collection periods, perfusate and dialysate samples were taken and stored at 4°C until they were analyzed for EtOH (<48 h from collection), as described previously (14). This fluorometric assay is based on the conversion of EtOH to acetaldehyde via alcohol dehydrogenase (ADH) and cofactor NAD. The fluorescent NADH product (excitation/emission at 360/415 nm) is directly proportional to EtOH in the original sample and was detected using the Victor3 Multilabel counter (Perkin-Elmer, Wellesley, MA). All samples were run in duplicate, measured against a standard curve, and averaged for data analysis. We calculated the in vivo EtOH O:I ratio as [EtOHdialysate]/[EtOHperfusate] to qualitatively estimate blood flow and calculate interstitial IL-15 and glycerol concentrations using the external standard approach. Since EtOH is

<table>
<thead>
<tr>
<th>Table 2. Basic blood chemistry for participants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lean</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
</tr>
<tr>
<td>HOMA</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
</tr>
</tbody>
</table>

Values are from resting blood drawn after an 8-h fast and are presented as means ± SD; n = 20 for all subjects combined (lean, n = 10; obese, n = 10). HOMA, homeostatic model assessment. *P < 0.05 vs. lean group. Blood chemistry values obtained from Unicel DxC 600i Synchron Access Clinical System (Beckman Coulter, Brea, CA). Details for calculated HOMA values are provided in MATERIALS AND METHODS.

Fig. 1. Study schematic of the experimental visit. During the preexercise/perfusion period, subjects remained supine in bed while IL-15 was perfused into subcutaneous adipose tissue (SCAT) in increasing concentrations during each successive stage. Subjects then cycled at the training heart rate [HR; using 60% HR reserve (HRR) and the Karvonen equation] for 1 h, followed by 1-h postexercise recovery. “MD sample” indicates period of time that dialysate was sampled for IL-15 and glycerol. “Blood” indicates where blood was sampled through intravenous (iv) catheter: MD, microdialysis; MD Eq., 60 min of equilibration before any MD sampling began.
not metabolized in the local tissue, EtOH is lost to the tissue in relation to the local microvascular flow. The resultant calculated ethanol O:I ratio is inversely related to local blood flow (14). See Interstitial concentration estimates for details on the interstitial estimates of IL-15 and glycerol.

**IL-15 assays.** IL-15 concentrations were measured in both SkM and SCAT dialysate and blood using a commercially available electro-

Chemiluminescent Ultra-sensitive IL-15 kit from Meso Scale Discovery (MSD; Rockville, MD). Briefly, sample medium was added to wells containing carbon electrodes precoated with IL-15 capture antibodies. After a labeled detection antibody and read buffer were added, the electrode was excited and the emitted light proportional to the amount of IL-15 in the sample. The signal was read using the MSD Sector 2400 and quantified using MSD Discovery Workbench software version 4. Plasma samples were run according to the manufacturer’s specifications without modifications. Dialysate and perfusate samples were measured in a modified protocol developed along with the manufacturer, where we prepared the calibration curve in a 0.9% saline and 1% BSA background. A small amount of BSA (1% final BSA) was added to the dialysate samples to standardize matrices, and we applied appropriate dilution factors to all unknown samples. Samples were run in duplicate where the volume allowed and averaged for data analysis. Intra- and interassay coefficient of variation was 6.21 and 8.41%, respectively.

**Interstitial concentration estimates.** To estimate interstitial IL-15 or glycerol concentrations, we first conducted in vitro experiments using the same probes and flow rates as in the in vivo/in situ conditions. The probes were placed in a beaker solution [Dulbecco’s phosphate-buffered saline, 0.1% bovine serum albumin (BSA), 5 mM glucose, 0.2 mM glycerol, 5 mM EtOH, 2 mM lactate, and varying concentrations of IL-15], similar to conditions described previously (23). The relationship between the in vitro recoveries of EtOH and a given substance of interest (e.g., IL-15) was used to calculate the in vivo interstitial concentration of the substance of interest, given the assumption that the relationship between the relative recoveries of two substances in vitro is the same as the relationship between the relative recoveries of those two substances in vivo. We used the following equation to calculate interstitial IL-15 ([IL15]:

\[
\text{[IL15]} = \left( \frac{\text{[EtOH]}_{\text{in vitro}}}{\text{[IL15]}_{\text{in vitro}}} \right) \times \left\{ \frac{\text{[IL15]}_{\text{in vivo}}}{1 - \text{EtOH O:I in vivo}} \right\}
\]

where [IL15] = measured in vivo dialysate IL-15, 1-EtOH O:I in vivo = in vivo EtOH lost to tissue from the perfusate, in vitro EtOH relative recovery = ([in vitro dialysate ethanol]/[in vitro beaker ethanol]), and in vitro IL-15 relative recovery = ([in vitro dialysate IL-15]/[in vitro beaker IL-15]). Over the several in vitro experiments conducted using the 100-kDa probes and a variety of beaker concentrations of IL-15, in vitro IL-15 recovery was ~3-6%, whereas calculated in vivo relative recoveries for IL-15 were 1.09 ± 0.32% for 15-mm dialysis membranes and 3.22 ± 1.03% for 30-mm membranes. In vitro relative recoveries for EtOH were ~85-98%, whereas calculated in vivo relative recoveries for EtOH were 32.7 ± 7.8% for 15-mm membranes and 61.2 ± 17.8% for 30-mm membranes. Interstitial IL-15 ([IL-15]) and interstitial glycerol ([Glycer]) concentrations were then calculated using the above equation.

**Statistical Analysis**

Most data analysis between lean and obese (demographics, blood measurements such as basic chemistries and resting IL-15, etc.) was assessed using a Student’s t-test. For differences examined across multiple time points (resting, exercise, recovery), we used a repeated-measures ANOVA. If a significant F-ratio was detected in the ANOVA models, we used a Fisher’s least significant difference post hoc analysis. To examine relationships between certain variables, we used Pearson Product Moment Correlation analysis. All statistical analyses were performed using SPSS Statistics for Windows version 20.0 (IBM, Armonk, NY). Data is reported as means ± SD, unless otherwise noted, and α-level was set at P ≤ 0.05.

**RESULTS**

**Resting Conditions**

**Physical characteristics.** The two distinct lean and obese BMI groups (lean: 23.1 ± 1.9 vs. obese: 34.7 ± 3.5 kg/m²; P < 0.01) were not significantly different in age (P = 0.30) and had a nearly equal distribution of men and women in each group (lean males/females 5:5, obese males/females 6:4). This BMI stratification also yielded expected differences in DEXA-assessed body composition parameters, as seen in Table 1. With regard to body fat distribution, obese individuals had a significantly higher percent body fat (P = 0.02) but also carried more fat in their upper body, as suggested by a higher android (upper body) percent fat (P < 0.01) and android/gynoid (upper body/lower body) fat ratio (P = 0.02).

**Blood chemistry.** Despite some large magnitude differences in mean concentrations, most of the blood chemistry measures were not found to be statistically different between lean and obese due to considerable variation (Table 2). However, the obese group demonstrated twofold higher insulin concentrations and HOMA scores (P < 0.01) than their lean counterparts. There were no differences in 8-h-fasted glucose between lean and obese subjects (P = 0.19).

**IL-15 concentrations.** We did not find a significant difference in resting circulating (plasma) IL-15 between lean and obese (P = 0.47). Furthermore, despite speculation that obese SkM would secrete less IL-15, we found that SkM IL-15 was also not significantly different between lean and obese for either dialysate IL-15 (dIL15; P = 0.86) or calculated interstitial IL-15 ([IL15]; P = 0.67) concentrations. Alternatively, we did observe a higher resting SCAT IL-15 in obese than lean both for dIL15 (P = 0.02) and iIL15 (P = 0.02) concentrations (Fig. 2). Although resting SCAT iIL15 was higher in obese subjects, resting plasma IL-15 was not significantly related to either resting SkM or SCAT iIL15 (Table 3) analyzed as a whole group (SkM vs. plasma IL-15: P = 0.17; SCAT vs. plasma IL-15: P = 0.92) or within each of the lean (SkM vs. plasma IL-15: P = 0.23; SCAT vs plasma IL-15: P = 0.85) or obese (SkM vs. plasma IL-15: P = 0.44; SCAT vs. plasma IL-15: P = 0.84) groups analyzed separately.

**IL-15 and SCAT glycerol relationships.** Resting calculated iGlycer was higher in obese than in lean subjects (obese: 307.7 ± 164.2 vs. lean: 175.5 ± 57.5 μmol/l, P = 0.03). Resting relationships between SkM/SCAT iIL15 and SCAT iGlycer can be seen in Fig. 3, with lean and obese plotted by different symbols but with one regression line [±95% confidence interval (CI)] for the entire group. Resting SkM iIL15 was not significantly related to SCAT iGlycer (Fig. 3A) when analyzed either as a whole group (r = 0.01, P = 0.97) or within each of the lean (r = –0.13, P = 0.72) or obese (r = 0.21, P = 0.59) groups analyzed separately. The amount of SCAT iIL15 was significantly related to the amount of SCAT iGlycer (Fig. 3B) when the group was analyzed together (r = 0.45, P = 0.05) but not when the lean (r = 0.47, P = 0.17) or obese (r = 0.25, P = 0.49) groups were analyzed separately.

**EtOH O:I ratio.** The resting EtOH O:I ratio was not different between lean and obese groups in SkM (lean vs. obese, P =
0.46) or in SCAT (lean vs. obese, $P = 0.10$), suggesting that there were no qualitative blood flow differences in a given tissue between lean and obese.

**Exercise Conditions**

*Exercise-induced plasma IL-15.* Figure 4A shows plasma IL-15 throughout the 60-min aerobic exercise bout and up to 1-h into recovery. A significant main time effect ($P < 0.01$) and post hoc tests indicated that in both lean and obese groups, exercise resulted in an increased circulating IL-15 during (30 min) and at the cessation of exercise (60 min) and that plasma IL-15 returned to baseline within 1-h of recovery. There was a BMI × time interaction ($P < 0.01$), but post hoc testing did not reveal any differences between lean and obese in plasma IL-15 at any time. However, the obese group demonstrated a greater relative increase from their preexercise to their average exercise value (obese: $20.3 \pm 10.8$ vs. lean: $10.8 \pm 7.5\%$ change, $P = 0.03$). Furthermore, the obese also demonstrated a higher total amount of IL-15 over the 60-min exercise bout (obese: $9.5 \pm 4.1$ vs. lean: $4.5 \pm 2.4$ pg·ml$^{-1}$·60 min, $P < 0.01$), indicated by the area under the curve analysis in Fig. 4B.

*Exercise-induced SkM IL-15.* Although there was an increased plasma IL-15 in lean and obese during compared with before exercise, there were no increases in SCAT iIL15 during exercise in lean or obese (time main effect, $P = 0.41$). Furthermore, there were no differences between the lean and obese groups in SCAT iIL15 when expressed as a raw concentration change (lean vs. obese SCAT iIL15 change, $P = 0.35$) or percent change (lean vs. obese SCAT iIL15% change, $P = 0.97$).

*Exercise-induced SCAT glycerol.* As a result of exercise, we observed an expected increase in SCAT iGlyc in both lean and obese groups (resting iGlyc: $200.4 \pm 50.9$ vs. exercise average iGlyc: $404.5 \pm 156.8$ μM; time main effect, $P < 0.01$); however, there were no differences between the two groups (BMI main effect, $P = 0.66$; BMI × time interaction, $P = 0.85$). Additionally, there were no differences between lean and obese groups in exercise-induced SCAT iGlyc when expressed either as a raw concentration change (lean vs. obese SCAT iGlyc raw change, $P = 0.85$) or percent change (lean vs. obese SCAT iGlyc %change, $P = 0.45$) from resting values.

*Exercise-induced EtOH O:1 ratio.* There was no statistical difference in the exercise-induced change in SCAT blood flow (indicated by the EtOH O:1 ratio) in either lean or obese subjects (main time effect, $P = 0.07$), nor did this response appear to be

---

**Table 3. Resting local and systemic IL-15 relationships**

<table>
<thead>
<tr>
<th></th>
<th>SkM iIL-15 vs. Plasma IL-15</th>
<th>SCAT iIL-15 vs. Plasma IL-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>$r = -0.33, P = 0.17, n = 19$</td>
<td>$r = 0.03, P = 0.92, n = 20$</td>
</tr>
<tr>
<td>Lean</td>
<td>$r = -0.42, P = 0.23, n = 10$</td>
<td>$r = -0.07, P = 0.85, n = 10$</td>
</tr>
<tr>
<td>Obese</td>
<td>$r = -0.30, P = 0.44, n = 9$</td>
<td>$r = -0.08, P = 0.84, n = 10$</td>
</tr>
</tbody>
</table>

SkM, skeletal muscle; SCAT, subcutaneous adipose tissue; iIL-15, interstitial IL-15. Resting relationships between local (SkM and SCAT) interstitial sources of IL-15 (iIL-15) and circulating IL-15. As indicated, there are no significant relationships ($P \geq 0.17$ for all analyses) between local and systemic IL-15.
dependent on BMI (BMI main effect, $P = 0.29$; BMI $\times$ time interaction, $P = 0.53$). There were also no differences observed between lean and obese groups in the exercise-induced EtOH O:I ratio when expressed as a raw change ($P = 0.53$) or percent change ($P = 0.49$) from resting values.

**Exercise-induced IL-15 and SCAT glycerol relationships.** Exercise-induced (%change) relationships between plasma IL-15 and SCAT iGlyc and between SCAT iIL15 and SCAT iGlyc can be seen in Fig. 5. Although the obese demonstrated an augmented increase in plasma IL-15 vs. their lean counterparts during exercise, there were no significant relationships observed between the exercise-induced plasma IL-15% change and SCAT iGlyc percent change (Fig. 5A) analyzed by the whole group ($r = -0.13$, $P = 0.59$) or within each of the lean ($r = 0.16$, $P = 0.71$) and obese ($r = -0.29$, $P = 0.48$) groups analyzed separately. Likewise, we did not observe any significant relationships between exercise-induced SCAT iIL15% change and SCAT iGlyc percent change (Fig. 5B) analyzed either within the whole group ($r = 0.21$, $P = 0.45$) or within each of the lean ($r = 0.09$, $P = 0.85$) or obese ($r = 0.41$, $P = 0.32$) groups analyzed separately.

**IL-15 Perfusion Conditions**

**SCAT glycerol.** In this part of the investigation, we aimed to perfuse IL-15 directly into SCAT tissue at physiologically relevant up through pharmacological concentrations to assess lipolytic responses to IL-15. As mentioned above, resting calculated iGlyc was higher in the SCAT of obese subjects than in the SCAT of lean subjects ($P = 0.03$). SCAT IL-15 perfusion (Fig. 6) resulted in a significant BMI $\times$ time interaction ($P = 0.02$) with regard to the SCAT iGlyc (lipolytic) response. In lean subjects, there was a 13.7% increase ($P = 0.04$) in iGlyc between the two lowest IL-15 perfusion concentrations (20 and 650 pg/ml), suggesting that IL-15 increased lipolysis in adipose tissue of lean subjects. By the next highest concentration (6,500 pg/ml), iGlyc concentrations were no longer elevated in the lean subjects and were similar to baseline for the remainder of the IL-15 perfusion period ($P > 0.05$). To the contrary, SCAT IL-15 perfusion decreased iGlyc by $-18.8\%$ between the resting (stage 0) and lowest IL-15 perfusion concentration (20 pg/ml) in obese subjects ($P < 0.01$), suggesting that IL-15 suppressed SCAT lipolysis in obese. The iGlyc concentrations remained suppressed and were still below baseline values ($P = 0.01$) at the end of the IL-15 perfusion period (65,000 pg/ml). Expressed as a percent change from baseline (stage 0, perfused with 0 pg/ml), the iGlyc change from 0 to 20 pg/ml perfusion was nearly different between lean and obese ($P = 0.07$), but the iGlyc change from 0 to 650 pg/ml perfusion was statistically different between lean and obese ($P = 0.03$).

**EtOH O:I ratio.** SCAT IL-15 perfusion did not alter the SCAT EtOH O:I ratio (SCAT blood flow), as evidenced through our observation of no main (time, $P = 0.12$; BMI, $P = 0.12$) or interaction effects (time $\times$ BMI interaction, $P = 0.87$) throughout the IL-15 perfusion protocol.

**DISCUSSION**

An IL-15 endocrine-like axis has been suggested to exist, in which SkM secretion of IL-15 to the blood could reach and have effects in adipose tissue; however, the most concrete
evidence that this axis exists comes from animal and cell culture experimental models (3, 6, 7, 27–29). Using blood and microdialysis sampling techniques, the current study provides the first data in humans of IL-15 in SkM and SCAT and their potential effects on adipose tissue metabolism. The primary findings were the following: 1) there was no difference in circulating IL-15 or SkM IL-15 between distinct lean and obese groups, 2) obese SCAT contained more IL-15 than lean SCAT, 3) the two local IL-15 sources (SkM and SCAT) examined did not appear to be related to systemic IL-15, 4) there was a significant positive correlation between SCAT IL-15 and SCAT lipolysis at rest, 5) there were expected rises in SCAT lipolysis during 60 min of aerobic exercise in both lean and obese, but the degree to which lipolysis increased was not related to the amount of IL-15 released during exercise, and 6) IL-15 seems to at least partially modulate lipolysis in human SCAT. Although our data do not support the proposed IL-15 endocrine axis in humans, we present data that suggest that IL-15 in SCAT tissue may serve a more important autocrine/paracrine role in humans (adipose tissue IL-15 acting in adipose tissue) rather than an endocrine role (SkM IL-15-blood-adipose tissue).

We first aimed to determine the amount of IL-15 in SkM and SCAT of lean and obese individuals and the degree to which interstitial IL-15 in SkM and SCAT was correlated with SCAT lipolysis. At least two studies had demonstrated previously that plasma IL-15 was higher in lean compared with obese subjects (6, 21). Those studies speculated that the reason for a higher systemic IL-15 in lean individuals was a higher production and secretion of IL-15 from SkM compared with SkM in obese. In fact, one of those studies reported no association between SkM IL-15 mRNA and plasma RNA, suggesting that SkM may not be the main source of a lower plasma IL-15 in humans (21). Through a direct assessment of IL-15 using in situ microdialysis and blood samples, we found no difference in resting SkM IL-15 or plasma IL-15 between lean and obese subjects. In contrast, we observed a higher interstitial SCAT IL-15 in obese than in lean subjects, which was not completely surprising given the proinflammatory nature of IL-15 (10, 11) and that obesity is considered a low-grade inflammatory condition (9, 31). Based on the magnitude of difference between SCAT and plasma IL-15, suppositions that IL-15 would reach adipose tissue from the circulation, operating as an endocrine mediator of body composition, do not seem likely. We contend that an autocrine/paracrine IL-15 axis (IL-15 released in adipose tissue acting in adipose tissue) is more likely to exist than the proposed SkM-blood-adipose tissue IL-15 axis, in line with a similar model identified in porcine adipocytes (1, 2). However, because the positive resting correlation between SCAT IL-15 and SCAT glycerol is not causal, it is very possible that IL-15 may not play a substantial role in regulating basal lipolysis in human adipose tissue. Thus, the current resting (basal/nonperfusion) lipolytic data should be taken with some caution.

Similar to previous reports, we demonstrated that exercise could increase plasma IL-15 (8, 26, 33); however, we are the first to show that plasma IL-15 increased to a greater extent in obese compared with lean subjects. Because we were not able to leave the microdialysis probes in SkM during exercise, due to the relatively fragile large pore size membrane we used for IL-15 sampling, it is difficult to rule out the possibility that changes in SkM IL-15 secretion could account for changes observed in plasma IL-15. However, even if SkM IL-15 is released to the blood during exercise, our observation that plasma IL-15 changes during exercise were not positively

![Fig. 5. Exercise-induced relationships between IL-15 and glycerol. Relationships are between the exercise-induced %change in plasma IL-15 and the exercise-induced %change in SCAT interstitial glycerol (n = 16; A) and between exercise-induced %change in SCAT interstitial IL-15 and exercise-induced %change in SCAT interstitial glycerol (n = 15; B). Solid lines are trend lines for the entire group, and dotted curved lines are ± 95% CI for entire group.](image1)

![Fig. 6. SCAT interstitial glycerol with IL-15 perfusion. MD probes were placed in the SCAT and perfused with various IL-15 concentrations to assess lipolytic responses in lean (n = 10) and obese (n = 10) subjects. Values on the y-axis are the calculated SCAT interstitial glycerol for each IL-15 perfusion stage and are presented as means ± SD. *P < 0.05 vs. preceding perfusion stage within BMI group.](image2)
correlated with changes in lipolysis during exercise suggests that IL-15 produced in and secreted from SkM would probably not alter fat mass via lipolysis in SCAT during exercise. Furthermore, without any clear direction in SCAT IL-15 exercise-induced changes, we believe that SCAT IL-15 is not a major contributor to the elevations in plasma IL-15 during exercise, although the study of more subjects or perhaps different methodology could clarify this point. Regardless of the source, we believe that acute exercise-induced changes in lipolysis most likely occur through other well-established lipolytic agents such as catecholamines and ANP (4, 18) and not IL-15.

Whereas previous investigations have reported IL-15-mediated metabolic actions in adipose tissue, IL-15 tissue exposures in these studies represent conditions rarely seen in vivo (2, 6, 28). To better study physiological actions of IL-15, we assessed direct effects of IL-15 perfusion on human adipose tissue using a wide range of concentrations and found that IL-15 was capable of increasing lipolysis in lean human adipose tissue. We also observed that the obese showed a suppression of lipolysis upon IL-15 exposure. Importantly, the direct IL-15-mediated changes in lipolysis were observed at perfusate concentrations likely to be seen by adipocytes in vivo. Based on an in vivo IL-15 relative recovery across the membrane, we estimate that lean and obese SCAT interstitial IL-15 was 11.8–40.3 pg/ml (95% CI) in lean SCAT and 33.5–123.5 pg/ml (95% CI) in obese SCAT. It is assumed that the same fraction of IL-15 was able to cross the membrane from the perfusate into the tissue during the IL-15 perfusion. Our measured IL-15 perfusate concentrations during stages 1–3 ranged from 20-6,500 pg/ml, which would represent SCAT IL-15 exposure between 0.3 and 91 pg/ml. Compared with the calculated interstitial concentrations, these exposure concentrations are likely in vivo physiological conditions and thus represent practical in vivo responses. Using the same estimates given the observed in vivo recovery, it is also possible that the perfused IL-15 concentration was at times actually lower than the prevailing interstitial concentrations and therefore did not elevate interstitial IL-15 concentrations. To better understand the potential in vivo role that IL-15 could have in altering lipolysis, further experiments would be required to verify the true interstitial concentrations and to calculate the amount of IL-15 to which SCAT tissue is exposed (e.g., diffusion of IL-15 into the tissue surrounding the probe) at a given IL-15 perfusate concentration.

Based on the lipolytic induction via IL-15 reported to occur in porcine adipocytes (2), we hypothesized that lean and obese SCAT would undergo an increased lipolysis with IL-15 exposure and that obese individuals would demonstrate a blunted response to IL-15 perfusion compared with lean individuals, as shown previously with catecholamines (16, 17). Given the large amount of fat tissue in obese individuals, it is thought that this blunted response is protective in nature (16). In opposition to our original hypothesis, we observed an increased lipolysis at a relatively low IL-15 concentration in the lean subjects, which was later decreased with higher concentrations, and a suppressed lipolysis in the obese with the lowest IL-15 perfusion concentration. Whether or not lipolytic suppression induced by IL-15 is a potential cause or consequence of obesity remains to be clarified.

**Limitations and Future Directions**

Although there are apparent differences in the metabolic (lipolytic) responses between lean and obese individuals attributed to IL-15, the biological significance of this IL-15 local axis with regard to human obesity remains to be clarified. As discussed previously, observations of IL-15 impacting lipolysis through microdialysis perfusion could be considered difficult to reconcile based on perfusate and interstitial concentrations and given our estimated IL-15 in vivo recovery. At the same time, we cannot exclude the possibility that our in vivo recovery estimates based on the in vitro and in vivo relative recoveries of IL-15 and ethanol might be underestimates of probe recovery or that unaccounted factors (e.g., the IL-15R subunit, which can exist as a soluble receptor) could also modify local/systemic IL-15 concentrations substantially. Thus, there may be more precise methods of measuring muscle and fat IL-15 (both protein expression and production rates) than microdialysis. Regardless, if IL-15 is indeed important for human body composition changes, future studies may be needed to examine other metabolic mechanisms of decreased fat mass with IL-15 exposure, such as reduced lipid uptake and deposition and increased fatty acid oxidation in human tissues, as well as the specific receptor subunit expression in human adipose tissue driving IL-15 biological responses. Until these further studies are conducted that confirm/extend our findings and the potential impact of IL-15 on human adipose tissue metabolism, the current data should be taken with caution. Finally, examining the true potential of IL-15 to mediate adipose tissue metabolism and body composition from a clinically relevant application would require human pharmacokinetic studies on the effects of IL-15 to support these claims. Such studies may be of interest to clinicians given ongoing clinical trials, using IL-15 as a chemotherapeutic agent.

**Conclusions**

There is a plethora of literature supporting an IL-15 endocrine axis (SkM-blood-adipose tissue) in the reduction of fat mass in cell culture and animal models, yet the current study does not support the existence of a similar axis in humans. Our data suggest that an uninvestigated IL-15 autocrine/paracrine axis might exist in human adipose tissue (e.g., SCAT IL-15 produced in and acting on SCAT), where it appears that IL-15 at certain doses can induce lipolysis in lean individuals and can suppress lipolysis in obese individuals. The collective findings herein further support links between obesity and increased proinflammatory molecules in SCAT (9, 20, 24, 25). How these specific increases in IL-15 and associated lipolytic responses in obese SCAT contribute to obesogenic and related comorbidities remains to be determined.

**ACKNOWLEDGMENTS**

We sincerely appreciate our subjects, who gave their time and efforts while participating in this study. We thank Patricia Brophy and Dustin Gooden for their assistance during the experimental visits and acknowledge Drs. Joseph Houmard, Jacques Robidoux, Carol Witczak, and David Brown for their advisory assistance and support during the dissertation process.

**GRANTS**

This work was conducted using facilities of the East Carolina University Diabetes and Obesity Institute and with partial funding support from a 2012 American College of Sports Medicine Foundation Doctoral Student Grant.
awarded to J. R. Pierce. R. C. Hickner was supported by National Institute of Diabetes and Digestive and Kidney Diseases R01 award DK-071081.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


