Impaired Suppression of Plasma Lipid Extraction and its Partitioning Away from Muscle by Insulin in Humans with Obesity

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Trial Registration: ClinicalTrials.gov (NCT01860911)

Abstract

Context: Humans with obesity and insulin resistance exhibit lipid accumulation in skeletal muscle, but the underlying biological mechanisms responsible for the accumulation of lipid in the muscle of these individuals remain unknown.

Objective: We investigated how plasma insulin modulates the extraction of circulating triglycerides (TGs) and non-esterified fatty acids (NEFAs) from dietary and endogenous sources in the muscle of lean, insulin-sensitive humans (Lean-IS) and contrasted these responses to those in humans with obesity and insulin resistance (Obese-IR).

Methods: The studies were performed in a postprandial state associated with steady-state plasma TG concentrations. The arterio-venous blood sampling technique was employed to determine the extraction of circulating lipids across the forearm muscle before and after insulin infusion. We distinguished kinetics of TGs and NEFAs from dietary sources across muscle from those from endogenous sources by incorporating stable isotope-labeled triolein in ingested fat.

Results: Plasma insulin rapidly suppressed the extraction of plasma TGs from endogenous, but not dietary, sources in the Lean-IS, but same response was absent in the Obese-IR. Furthermore, in the muscle of Lean-IS, plasma insulin decreased the extraction of circulating NEFAs from both dietary and endogenous sources, but in Obese-IR subjects this response was absent for NEFAs from dietary sources.

Conclusions: Partitioning of circulating lipids away from the skeletal muscle when plasma insulin increases, such as during the postprandial period, is impaired in humans with obesity and insulin resistance.

Key Words: triglycerides, insulin resistance, muscle, obesity, stable isotopes, forearm

Humans with obesity and insulin resistance display accumulation of lipids in skeletal muscle (1,2,3). The disposal of circulating lipids into the muscle is an important factor in the equation that determines lipid accumulation in muscle. Although elevated fasting-state circulating NEFAs have been proposed to drive lipid accumulation into the muscle of humans with obesity and insulin resistance (4,5), several lines of evidence argue against this notion (6,7,8). Specifically, plasma NEFA extraction, but not plasma NEFA concentration, is associated with insulin resistance in muscle of humans with obesity (8). Circulating TGs constitute a source of fatty acids taken by the muscle, and current evidence shows that muscle takes up a substantial portion, if not all, of the fatty acids liberated following extraction of plasma TGs (9,10). This response is of particular importance in the postprandial period (i.e., fed state) because both ingested fat-containing-TG (i.e., dietary TG) and endogenous fat-containing-TG are present in the circulation. Postprandial lipid metabolism is complex, and it has been a challenge to delineate between favorable and unfavorable responses, particularly in regards to how ingested fat is handled during the postprandial period (11). A key step for to the uptake of fatty acids liberated from circulating TGs is the extraction of the circulating TGs by the action of endothelial LPL in muscle capillaries.

Previous research evaluating the extraction of circulating TGs by the muscle show that humans with insulin resistance have increased uptake of plasma TGs during the postprandial period (12,13). Because the gene expression of muscle LPL does not change during the postprandial period or differs as function of the insulin-resistant state, the observed responses cannot be attributed to changes in the LPL gene expression in muscle (13). However, changes in the activity, as opposed to gene expression, of intravascular LPL in muscle may explain differences in these responses. Hormonal and nutritional stimuli are primary regulators of LPL activity (14,15), and can hence control the extraction of circulating TGs during the postprandial period. The increase in

plasma insulin is a key hormonal response of the postprandial period, and prolonged (i.e., ~ 4 hours) infusion of insulin in healthy subjects decreases the activity of LPL in muscle (16). Such evidence implies that the extraction of plasma TGs would be suppressed in muscle when plasma insulin increases. Moreover, insulin sensitivity correlates directly with the insulin-mediated decrease in muscle LPL activity (16), suggesting that the possible physiological effect of plasma insulin on suppressing the extraction of plasma TGs in muscle would be less evident in the presence of insulin resistance. Further, it is likely that suppression of plasma TG extraction by plasma insulin is evident particularly on TGs carrying dietary fat because of a preferential extraction of circulating TGs containing ingested fat during the postprandial period (10).

The importance of employing appropriate experimental techniques to differentiate between endogenous and dietary lipid extraction by the muscle, as well as how insulin action and resistance to the insulin action affect muscle lipid metabolism, have all been previously reviewed (6,17). Failure of plasma insulin to suppress extraction of lipids by the muscle in individuals with obesity and insulin resistance can result in significant accumulation of lipids into the muscle, secondary to increased disposal of lipid into the muscle and because of the recurrent nature of the postprandial state in the course of the day. Furthermore, because LPL acts preferentially on circulating TGs from dietary sources (10) and because it is known that the fatty acid composition of lipids stored in the skeletal muscle reflects that of the diet (18), it is important to distinguish between the extraction of circulating lipids from dietary sources and endogenous sources.

Previous research has used the arterio-venous (A-V) sampling technique to evaluate the extraction of circulating TGs in the postprandial state in the muscle of humans with obesity in conjunction with meal ingestion that results in non-steady state plasma TG concentrations (12,13). Because there is a rather non-negligible transit type of the metabolites across tissues, there are

methodological limitations when applying the A-V sampling technique under non-steady state metabolite concentrations (19,20). Also, higher plasma insulin concentrations in the postprandial state in humans with obesity and insulin resistance can mask physiological impairments related to substrate metabolism in insulin resistance, because such responses can be overcome by the greater insulin levels in the plasma of the humans with obesity (21). To this end, we conducted a mechanistic study employing a carefully-designed experimental protocol to determine how acute increase in plasma insulin affects the extraction of plasma TGs, which reflects LPL activity, in muscle of humans with obesity and insulin resistance, and compared these responses with those observed in lean, insulin sensitive humans. We used dietary fat labeled with a stable isotope tracer in order to discern the specific effects of plasma insulin on regulating the extraction of lipids originating from ingested fat.

Materials and Methods

Study design

Seventeen volunteers, ages 18 to 40 years, took part in a study to quantify the fractional extraction of circulating lipids across the forearm muscle. To quantify the extraction of lipids derived specifically from dietary fat, subjects ingested whipping cream enriched with ^{13}C-triolein. We determined muscle extraction of dietary and endogenous plasma TGs and NEFAs, along with that of plasma glucose, at fasting plasma insulin concentrations (Basal Study Period) and during increased plasma insulin concentrations (Insulin Study Period; Figure 1). The A-V sampling technique was employed to determine the extraction of circulating substrates across the forearm muscle. Blood samples were collected from the radial artery and a deep vein draining the forearm muscle while the study participants laid on bed for the duration of the experiment.

The study was conducted according to Declaration of Helsinki principles and all study procedures were carried out after obtaining approval from the Institutional Review Board at Mayo Clinic (IRB # 12005173). Prior to participation, each study participant gave written, informed consent. The study was registered with ClinicalTrials.gov (NCT01860911).

Study participants

Initial screening of interested participants was performed over the phone. Individuals were excluded from the study if they reported diabetes, history of abnormal lipid metabolism, medications, smoking, current participation in a weight-loss regimen, extreme dietary practices, took supplements known to affect the vasculature or lipid metabolism (i.e., cocoa, arginine, protein, fish oil), used anabolic steroids or corticosteroids, or participated in physical activity more than two days per week. Individuals that met the initial criteria were invited for further screening in order to estimate their insulin sensitivity if they had body mass index (BMI) ≤ 25 kg/m^2 (i.e., lean subjects) or BMI ≥ 32 kg/m^2 (i.e., subjects with obesity).

Screening procedures

For the screening visit, participants arrived in the Clinical Studies Infusion Unit (CSIU) at Mayo Clinic in Scottsdale, Arizona, in the morning after an overnight fasting period (i.e., 12 hours). The purpose of the study, the exact experimental procedures, and the associated risks were discussed with each participant prior to obtaining written consent. Screening procedures included medical history, standard physical examination, electrocardiogram (ECG), routine blood tests, and urinalysis. Subjects were excluded from the study if there was evidence of acute illness, metabolic disease such as liver or renal disease, atrial fibrillation, history of syncope, limiting or unstable

angina, congestive heart failure, ECG-documented abnormalities such as >0.2 mV ST-segment depression and frequent arrhythmias (>10 premature ventricular contractions/min), low hemoglobin or hematocrit (i.e., lower than accepted laboratory values), elevated blood pressure (systolic, >140 mmHg or a diastolic, >95 mmHg), plasma TG concentration >2.3 mmol/l, or 2-h plasma glucose > 11.1 mmol/l during an oral glucose tolerance test (OGTT). Insulin sensitivity was estimated from the responses of the plasma glucose and insulin concentrations during the OGTT, and by calculating the Matsuda Insulin Sensitivity Index (ISI) (22). We studied humans with obesity and insulin resistance (Obese-IR) and lean, insulin-sensitive humans (Lean-IS) with Matsuda ISI < 4.5 and > 7.0, respectively. We studied groups of subjects with distinct differences in insulin sensitivity in order to minimize variability in the responses resulting from biological and technical confounding factors. This enhances our ability to detect differences in the regulation of circulating lipids across muscle by plasma insulin that result from differences in insulin sensitivity. Body composition, including distinct components of body lean and fat tissues (i.e., visceral fat, forearm fat), were evaluated using Dual-energy X-ray absorptiometry (DEXA).

Infusion study visit

Participants arrived at the CSIU for the infusion study visit at ~ 7 am after fasting for ~12 hours. Subjects were asked to maintain their typical diet starting three days prior to the infusion day, and avoid any beverages with alcohol, as well as any form of physical activity during the same period beyond their normal daily physical activities. Compliance with these instructions was verbally verified when participants arrived at the CSIU, and prior to the initiation of the experimental procedures. An intravenous (IV) line was placed in a retrograde fashion deep in an antecubital vein draining the forearm muscle. To ensure that the selected vein drains primarily

deep forearm muscle, oxygen saturation in the blood drawn from this IV line had to be <60% (23); if not, the IV line would be repositioned. Another catheter was placed under local anesthesia in the radial artery of the opposite arm for arterial blood sampling. In addition to the arterial catheter, an additional IV line was inserted in an antecubital vein of the same arm for infusion of insulin. All catheters were kept patent by continuous infusion of saline.

A depiction of the experimental protocol is shown in Figure 1. Background blood samples were obtained at time 0, prior to ingestion of whipping cream containing (per 15 ml) 5 g of fat, 1 g of carbohydrate, and 0 g of protein. Whipping cream was enriched (~4%) with [1,1,1-$^{13}C_3$, 99%]triolein (Cambridge Isotope Laboratories, Inc., MA). Labeled triolein was incorporated into the whipping cream immediately prior to each experiment by sonication for five mins. After adding a small amount of artificial sweetener, the whipping cream was refrigerated until it was consumed. Subjects ingested a priming bolus of whipping cream [200 mg fat·kg^{-1}fat free mass (FFM)], followed by ingestion of smaller boluses starting 2 hours later and every 15 mins thereafter until the end of infusion study (Figure 1), resulting in an overall fat ingestion of 100 mg fat·kg FFM^{-1}·h^{-1} during the course of the experiment. We employed an experimental design that involves ingestion of small boluses of fat in order to avoid increase in the concentration of plasma TGs carrying ingested TG. Lipoprotein particles carrying ingested TG have 50-fold higher affinity for LPL than lipoproteins carrying endogenous TG (24), and in a way that can independently inhibit clearance of lipoproteins carrying endogenous TG by substrate competition for LPL (25). Therefore, by studying the plasma TG metabolism across muscle with no perturbation in the plasma TG concentrations and experimentally manipulating a single variable (i.e., plasma insulin concentration), our experimental approach allowed isolating the specific role of plasma insulin in regulating the extraction of circulating TG across muscle. The overall duration of the experimental

protocol was chosen to avoid recycling of ingested lipid in the endogenous plasma TG pool, and because it takes at least three hours after a meal for fat from that meal to appear in the endogenous TG pool (10,26). Consequently, the presence of [^{13}C]-Oleate in plasma lipids indicates the presence of dietary fat, making it possible to differentiate between ingested fat and endogenous fat in plasma lipid metabolism across the forearm muscle.

To determine the effects of increased plasma insulin concentrations on variables of interest, insulin was infused at a rate of 1.0 mU·kg^{-1}·min^{-1} (2.0 mU·kg^{-1}·min^{-1} for the first 10 mins) and 0.5 mU·kg^{-1}·min^{-1} (1.0 mU·kg^{-1}·min^{-1} for the first 10 mins) in the Lean-IS and Obese-IR subjects, respectively. Insulin was infused at a lower rate in the Obese-IR subjects, in order to achieve comparable absolute change in plasma insulin concentrations between the Obese-IR and Lean-IS subjects during the insulin infusion study period. This is because insulin resistant subjects have slower clearance of insulin from plasma, and lower insulin infusion rates in the Obese-IR subjects allowed for comparable absolute change in plasma insulin responses between groups during insulin infusion (27). If insulin were to be administered at the same rate across subjects, the Obese-IR subjects would have had much higher plasma insulin concentrations, which can mask responses of plasma lipid metabolism to plasma insulin. This is comparable to the situation where greater increase in plasma insulin concentrations in humans with obesity overcomes impaired plasma glucose metabolism in these individuals (21). Therefore, in contrast to previous research that evaluated plasma lipid metabolism in muscle in humans with obesity in the presence of variable plasma insulin concentrations induced by meal ingestion and which were higher in subjects with obesity (12,13), we quantified plasma lipid metabolism across muscle in the Obese-IR and Lean-IS subjects under comparable absolute change in the levels of plasma insulin. By utilizing the

particular study design, we can precisely characterize how plasma insulin controls the fractional extraction of lipids across muscle in obesity and insulin resistant state.

To maintain blood glucose concentrations at the basal levels during the insulin infusion, 20% dextrose was infused at a rate that was adjusted based on blood glucose concentration measurements performed every five mins (i.e., hyperinsulinemic-euglycemic clamp procedure). Two blood samples were collected five minutes apart at the end of the Basal and Insulin infusion study periods (Figure 1) to compare the effects of increased plasma insulin on the variables of interest. Arterial and venous blood samples were collected simultaneously from the radial artery line and the line draining the forearm muscle, respectively.

Blood flow

Branchial artery blood flow was measured using Doppler ultrasound in the Basal period and at the end of the 30-min Insulin infusion period in a group of (i.e., first seven) Lean-IS subjects, and by following procedures previously described (28). Branchial artery blood flow was measured by evaluating the branchial artery diameter and blood velocity using 2D Doppler ultrasound (CX50, Philips Medical Systems) and a linear array transducer placed over the branchial artery. We recorded two separate 2-minute segments, which were processed at a later time using specialized software. The following equation was used to compute blood flow (expressed as $ml \cdot min^{-1}$): Blood Flow = $v \cdot \pi \cdot (d/2)^2 \cdot 60$, where v = mean blood velocity ($cm \cdot s^{-1}$), $\pi = 3.14$, and d = arterial diameter (cm).

Analyses of plasma samples

Blood samples for the determination of plasma TGs and NEFAs were collected in tubes containing orlistat, which was added (i.e., 30 ug per ml of blood) to inhibit the in vitro lipolysis of TGs (29). All tubes were kept on ice until they were centrifuged for the collection of plasma samples, which were then stored at -80°C until analyses. An automated blood glucose analyzer (YSI 2300, Yellow Springs, OH) was used to measure the arterial blood glucose concentration during the infusion study in order to obtain feedback on how to adjust the infusion rate of 20% dextrose in response to the insulin infusion. Following the study, concentrations of plasma glucose in arterial and venous samples were determined by averaging measurements obtained in triplicate using the same glucose analyzer. Concentrations of plasma TG in the arterial and venous samples were determined enzymatically using a commercially available assay (Sigma-Aldrich, St. Louis, MO) and by calculating an average concentration based on ten replicates.

Analyses for the determinations of the percentage of oleate within the TG fraction (i.e, oleate-TG), ^{13}C enrichment of oleate-TG (i.e., [^{13}C]-oleate-TG), concentrations of individual NEFAs (i.e., myristate, palmitate, palmitoleate, palmitelaidate, stearate, oleate, elaidate, linoleate, α-linolenate, arachidonate, eicosapentaenoate, docosahexaenoate), and ^{13}C enrichment of oleate-NEFA (i.e., [^{13}C]-oleate-NEFA) in the arterial and venous plasma samples were performed at the Mayo Clinic Metabolomics Core. The analyses were performed on a liquid chromatography mass spectrometry (LC-MS) as previously described (30) using an Agilent 6460 triple quadrupole mass spectrometer coupled with a 1290 Infinity liquid chromatography system, allowing for high levels of analytical precision measurements. TGs were isolated by solid phase extraction as described by Bodennec et al. (31). Isolated TGs underwent basic hydrolysis and additional extraction prior to analysis on the LC-MS. Oleate-TG was expressed as a percentage of the sum of 12 individual fatty acids from the same fraction, while [^{13}C]-oleate-TG was expressed as tracer-to-tracee ratio (TTR).

Concentrations of NEFAs and TTR of [^{13}C]-oleate-NEFA in plasma were measured in aliquots that did not go through TG isolation. The concentration of total NEFAs in plasma was calculated as the sum of the measured concentrations of the individual plasma NEFAs indicated above.

The concentrations of plasma insulin (80-INSHU-E01.1; ALPCO Diagnostics, Windham, NH), and APO-CII and APO-CIII (Ab168549 and Ab154131, respectively, Abcam, Waltham, MA) in the arterial plasma samples were measured using commercially available ELISA assays.

Calculations

The concentration of oleate-TG in arterial and venous plasma samples was calculated by multiplying the measured percentage of oleate in the plasma TGs by the corresponding measured concentration of plasma TGs. The concentration of plasma [^{13}C]-oleate-TG was calculated by multiplying the [^{13}C]-oleate-TG TTR by the corresponding plasma oleate-TG concentration. The concentration of unlabeled-oleate-TG in plasma was calculated as the difference between the concentration of plasma oleate-TG and that of plasma [^{13}C]-oleate-TG. Arterial and venous plasma concentrations of [^{13}C]-oleate-NEFA were calculated by multiplying the [^{13}C]-oleate-NEFA TTR by the corresponding plasma oleate-NEFA concentration. The concentration of unlabeled-oleate-NEFA was calculated as the difference between the concentration of plasma oleate-NEFA and that of plasma [^{13}C]-oleate-NEFA.

We calculated the A-V concentration differences of the metabolites of interest across the forearm muscle, and where a positive A-V concentration difference of a given metabolite suggests metabolite uptake by the muscle, while a negative A-V concentration difference suggests release from the muscle (10). Fractional extraction of plasma TG across the forearm indicating intravascular lipolysis by LPL (32), was calculated as the A-V plasma TGs concentration

difference divided by the arterial plasma TG concentration, and expressed as a percentage (33). Fractional extraction of plasma NEFA across the forearm was calculated the same way.

Statistics

The normality of the data was evaluated using the Shapiro-Wilk test in conjunction with a visual examination of the corresponding Q-Q plots. A logarithmic transformation was applied to datasets that displayed a non-normal distribution prior to performing statistical analysis. Unpaired t-test was used to assess differences in subject characteristics. A two-way (time x group) repeated-measures analysis of variance (ANOVA) was employed to examine the main effects of plasma insulin concentrations and obesity/insulin-resistant state on the parameters of interest. Pairwise comparisons were performed using the Bonferroni multiple comparisons test. All tests, with the exception of those testing differences between groups for APO-CII and APO-CIII, were two-sided, and $P \leq 0.05$ was considered statistically significant. Data are reported as mean ± standard error of the mean (SEM). All statistical analyses were performed using GraphPad Prism Software (version 8.4.3). To enhance the interpretation of our results and to facilitate integration of our data in similar research, we employed a web-based effect size calculator (34) to compute observed effect size values (i.e., Cohen's *d*) for the study's primary end-points.

Results

Anthropometric and clinical characteristics of the Lean-IS subjects and the Obese-IR subjects are presented in Table 1. By design, the two groups differed in insulin sensitivity (see Methods). Clinical chemistry parameters reported in Table 1 were determined in blood samples collected during the screening visit and analyzed at the Mayo Clinical Laboratories at Mayo Clinic-Arizona.

Arterial Plasma Concentrations of Insulin, Glucose, Triglycerides, and NEFA

Insulin

Table 2 shows the concentrations of insulin and various metabolites in arterial plasma in the fasting state, as well as their corresponding concentrations in the Basal and Insulin infusion periods. Plasma insulin concentrations were higher in the Obese-IR than the Lean-IS subjects at Fasting ($P < 0.0001$) and at the Basal study period ($P < 0.05$). When compared to the Basal study period, plasma insulin concentrations increased in both the Lean-IS ($P < 0.0001$) and the Obese-IR ($P < 0.01$) subjects during the insulin infusion. As expected based on our study design for comparable absolute changes between groups in the concentrations of plasma insulin during the Insulin infusion period (see Methods) the delta change from Basal in the plasma insulin concentrations ($\mu IU \cdot ml^{-1}$) was not different between the Lean-IS and Obese-IR subjects (49 ± 3 vs 58 ± 9; $P = 0.35$).

Glucose

Plasma glucose concentrations were maintained at steady-state during the Basal and Insulin infusion study periods of the experiments (Table 2 and Figure 2B). This was expected because of the experimental protocol, which was designed to maintain the plasma glucose concentrations during the Insulin infusion period in each subject at the levels observed in the Basal period and via intravenous infusion of dextrose.

Triglycerides

ANOVA showed a significant main effect for group for the total TG concentration in arterial plasma, with the total plasma TG concentrations being higher in the Obese-IR subjects (Table 2). Post-hoc comparisons did not identify any differences in the total plasma TG concentrations between the Basal and the Insulin infusion periods within groups ($P > 0.05$). Similar results were obtained for the concentration of the unlabeled-oleate within the arterial plasma TG when comparing the Basal and Insulin infusion periods, and where ANOVA indicated main effects for group ($P = 0.05$), but no differences in the plasma unlabeled-oleate-TG concentrations were detected between the Basal and the Insulin infusion periods within groups ($P > 0.05$). No differences were found between groups or between the Basal and Insulin infusion periods within groups in the arterial concentrations of plasma [^{13}C]-oleate-TG (for all $P > 0.05$).

Non-esterified fatty acids

ANOVA results for the total NEFA concentrations in arterial plasma are shown in Table 2, indicating a main effect for the response to the increased plasma insulin concentrations. Total plasma NEFA concentrations in arterial plasma were lower in the Insulin infusion period compared to Basal in both the Lean-IS ($P < 0.001$) and the Obese-IR ($P < 0.05$) subjects, with the concentration of total NEFAs in arterial plasma being significantly lower in the Lean-IS than the Obese-IR subjects in the Insulin infusion period ($P < 0.01$). Similar results were obtained for the concentrations of unlabeled-oleate within the NEFAs in arterial plasma, and where the concentration of unlabeled-oleate-NEFAs in arterial plasma was lower in the Insulin infusion period compared to Basal period in both the Lean-IS ($P < 0.0001$) and the Obese-IR ($P < 0.001$) subjects. Also, the concentration of unlabeled-oleate-NEFA in arterial plasma was significantly lower in the Lean-IS than the Obese-IR subjects in the Insulin infusion period ($P < 0.01$). The

concentration of [^{13}C]-oleate-NEFA in arterial plasma was lower during the Insulin infusion period when compared to the Basal period in the Lean-IS ($P < 0.05$), but not in the Obese-IR ($P = 0.31$), subjects.

Isotopic enrichment

Triglycerides

ANOVA showed differences for time, but not for group, for both arterial and venous plasma [^{13}C]-oleate-TG enrichment (Table 3). Post-hoc pairwise comparisons indicated no differences in the Lean-IS subjects for plasma [^{13}C]-oleate-TG TTR in the Insulin infusion period when compared to the Basal period in either the artery ($P = 0.32$) or the vein ($P = 0.10$). However, in the Obese-IR subjects, and when compared to Basal, plasma [^{13}C]-oleate-TG TTR in the Insulin infusion period demonstrated a trend (P value < 0.10) toward being significantly higher in both the artery ($P = 0.08$) and vein ($P = 0.06$). No main effects or interaction were detected for the arterio-venous (A-V) difference for plasma [^{13}C]-oleate-TG TTR across muscle ($P > 0.05$).

Non-esterified fatty acids

ANOVA results for arterial and venous plasma [^{13}C]-oleate-NEFA enrichment are shown in Table 3. Post-hoc pairwise comparisons indicated that within the Lean-IS group, plasma [^{13}C]-oleate-NEFA TTR was higher in the Insulin infusion period compared to the Basal period in both the artery and the vein (for both $P < 0.0001$), and similar responses were observed for the Obese-IR group in both the artery and the vein (for both $P < 0.01$). The A-V difference for plasma [^{13}C]-oleate-NEFA TTR across the muscle showed main effects for group ($P < 0.05$), time ($P < 0.01$), and group x time interaction ($P < 0.01$). Specifically, and when compared to the Basal period, the

A-V difference for plasma [^{13}C]-oleate-NEFA TTR across the muscle in the Insulin infusion period increased in the Lean-IS (0.0008 ± 0.0005 vs 0.0043 ± 0.0010; $P < 0.001$), but not in the Obese-IR (0.0001 ± 0.0004 vs 0.0001 ± 0.0007; $P = 0.99$) subjects.

Forearm arterio-venous differences of metabolites

Glucose

ANOVA results for A-V differences in the concentrations of plasma glucose as well as unlabeled and labeled TGs and NEFAs across muscle are presented in Table 4. Post-hoc comparisons indicated that the A-V difference in the concentrations of plasma glucose increased significantly in response to the Insulin infusion in the Lean-IS ($d = 2.09$; $P < 0.001$), but not in Obese-IS ($d = 1.30$; $P = 0.10$) subjects.

Triglycerides

There was a main time effect for the A-V difference in plasma total TG concentrations in response to the Insulin infusion (Table 4). Post-hoc tests showed a trend for a significant decrease from Basal in the A-V difference in plasma total TG concentrations in the presence of increased plasma insulin in the Lean-IS ($d = -1.08$; $P = 0.09$), but not in the Obese-IR (d = -0.39; $P = 0.43$) subjects. Comparable responses were observed for the A-V difference in unlabeled oleate within the plasma TG pool, and where a trend for a significant decrease in the A-V difference in plasma concentrations of unlabeled-oleate-TG in the presence of increased plasma insulin was evident in the Lean-IS ($d = -0.93$; $P = 0.07$), but not in the Obese-IR ($d = -0.07$; $P = 0.99$) subjects. On the other hand, the A-V difference in plasma concentrations of [^{13}C]-oleate-TG in the presence of

increased plasma insulin was not different from that at Basal in either the Lean-IS or the Obese-IR subjects (for both $P > 0.05$).

Non-esterified fatty acids

The A-V difference in the plasma total NEFA concentrations decreased in response to the insulin infusion when compared to Basal and became negative (from positive in the Basal period) in both the Lean-IS (d = -1.27; P <0.0001) and the Obese-IR (d = -0.62; $P < 0.05$) subjects (Table 4). The delta decrease from Basal (i.e., Insulin infusion period value minus Basal period value) in the A-V difference for the plasma total NEFA concentrations ($\mu mol \cdot L^{-1}$) was greater in the Lean-IS (-62 ± 10) than the Obese-IR (-29 ± 11; d = 1.11; $P < 0.05$) subjects. The A-V difference in the concentrations of unlabeled oleate within the plasma NEFA pool paralleled that of the plasma total NEFAs and became negative (from positive in the Basal period) in both the Lean-IS (d = -1.14; P <0.0001) and Obese-IR (d = -0.73; $P < 0.05$) subjects. The delta decrease from Basal (i.e., Insulin infusion period value minus Basal period value) in the A-V difference for the unlabeled-oleate-NEFA concentrations ($\mu mol \cdot L^{-1}$) showed a trend towards being significantly greater in the Lean-IS (-25 ± 4) than the Obese-IR (-12 ± 4; d = 0.97; P = 0.06) subjects. The A-V difference in the concentrations of [^{13}C]-oleate within the plasma NEFA pool decreased and became negative (from positive in the Basal period) in the Lean-IS (d = -1.21; P <0.05), but not in the Obese-IR (d= 0.03; P = 0.99) subjects (Table 4). The delta decrease from Basal (i.e., Insulin infusion period value minus Basal period value) in the A-V difference for the [^{13}C]-oleate-NEFA concentrations ($\mu mol \cdot L^{-1}$) was greater in the Lean-IS (-0.240 ± 0.092) than the Obese-IR (0.004 ± 0.048; d = 1.11; $P < 0.05$) subjects.

Forearm fractional extraction of metabolites

Glucose

ANOVA results showed significant main effects for the plasma glucose fractional extraction across muscle for group ($P < 0.01$), time ($P < 0.0001$), and their interaction ($P < 0.05$). Multiple comparisons tests indicated that the plasma glucose fractional extraction (%) was not different between groups in the Basal Period (Lean-IS: 5.0 ± 0.8; Obese-IR: 2.6 ± 0.4; $P = 0.64$). The plasma glucose fractional extraction (%) across muscle increased significantly from Basal in response to the Insulin infusion in the Lean-IS ($d = 2.26$), but not in the Obese-IR ($d = 1.28$) subjects (Figure 2A). The delta increase from Basal (i.e., Insulin infusion period value minus Basal period value) in the plasma glucose fractional extraction in muscle was greater in the Lean-IS than the Obese-IR (13.1 ± 2.4 vs 4.9 ± 1.8; $d = 1.31$; $P < 0.05$) subjects.

Triglycerides

There were significant main effects for the plasma total TG fractional extraction (%) across muscle for group, time, and their interaction (for all $P < 0.05$). Post-hoc multiple comparisons tests indicated that the plasma total TG fractional extraction (%) in the Basal period was lower in the Obese-IR (3.3 ± 0.8) than the Lean-IS (8.8 ± 1.9; $d = 1.22$; $P < 0.05$) subjects. The fractional extraction (%) of plasma total TG across muscle decreased significantly from Basal in response to the Insulin infusion in the Lean-IS ($d = -1.18$; $P < 0.01$), but not in the Obese-IR ($d = -0.23$; $P > 0.05$) subjects. The delta decrease from Basal in plasma total TG fractional extraction (%) in muscle in response to the insulin infusion (i.e., Insulin infusion period value minus Basal period value) was greater in the Lean-IS than the Obese-IR (-5.3 ± 1.8 vs -0.5 ± 1.0; $d = 1.11$; $P < 0.05$) subjects.

Comparable results to that of the fractional extraction (%) of the plasma total TGs in muscle were obtained with respect to the fractional extraction (%) specifically of the unlabeled (i.e., endogenously produced)-oleate within the plasma TG pool. The fractional extraction (%) of unlabeled-oleate-TG across the muscle in the Basal Period was lower in the Obese-IR (4.0 ± 0.9) than the Lean-IS (9.9 ± 2.3) (d = 1.15; P < 0.05) subjects. The fractional extraction of unlabeled-oleate-TG across muscle decreased significantly from Basal in response to the insulin infusion in the Lean-IS (d = -3.31), but not in the Obese-IR (d = 1.22) subjects (Figure 2A). Also, the delta change from Basal in the fractional extraction (%) of unlabeled-oleate-TG across the muscle in response to the insulin infusion was greater in the Lean-IS than the Obese-IR (-6.6 ± 2.7 vs 1.2 ± 1.3; d = 1.23; P < 0.05) subjects.

In contrast to the lower fractional extraction of unlabeled-oleate-TG in the muscle of the Obese-IR subjects in the Basal period, the fractional extraction (%) of [^{13}C]-oleate-TG in muscle in the same period was not different between the Lean-IS and the Obese-IR subjects (29.5 ± 13.7 vs 22.0 ± 13.6; d = 0.18; P > 0.05). The fractional extraction (%) of plasma [^{13}C]-oleate-TG in the muscle did not change significantly from Basal in response to the Insulin infusion in either the Lean-IS (d = 0.13) or the Obese-IR (d = -1.51) subjects (Figure 2B). Also, the delta change from Basal in response to the insulin infusion in the plasma [^{13}C]-oleate-TG fractional extraction in the muscle was not different between the two groups (P > 0.05).

Non-esterified fatty acids

The fractional extraction of total plasma NEFAs (%) in the Basal period was not different between the Lean-IS and Obese-IR subjects (3.6 ± 4.2 vs 3.6 ± 3.8; d = 0.01; P > 0.05). In response to the insulin infusion, the fractional extraction of total plasma NEFAs decreased in both groups

and became negative (from positive) indicating a release of NEFAs from muscle. This response was significantly larger in the Lean-IS (d = -4.05; $P < 0.05$) than the Obese-IR (d = -2.25; $P > 0.05$) subjects. Accordingly, the delta change from Basal describing the fractional release (%) of plasma total NEFAs from muscle in response to the insulin infusion was greater in the Lean-IS than the Obese-IR subjects (-53.1 ± 14.8 vs -11.3 ± 3.0; d = 1.27; $P < 0.05$).

Similar findings to that of the fractional extraction of plasma total NEFA in the muscle were obtained with respect to the fractional extraction specifically of the unlabeled oleate within the plasma NEFA pool. In the Basal period, the fractional extraction (%) of plasma unlabeled-oleate-NEFAs in the muscle did not differ between the Obese-IR (5.5 ± 3.8) and Lean-IS (5.6 ± 4.9) subjects (d = 0.01; $P > 0.05$). In response to the insulin infusion, the fractional extraction of plasma unlabeled-oleate NEFAs decreased in both groups and became negative (from positive) indicating a release of plasma unlabeled-oleate NEFAs from muscle, and this response was significant in both the Lean-IS (d = -3.81) and the Obese-IR (d = -2.39) subjects (Figure 4A). However, the delta change from Basal describing the fractional release (%) of plasma unlabeled-oleate NEFAs from muscle in response to the insulin infusion was greater in the Lean-IS than the Obese-IR (-64.3 ± 19.9 vs -12.7 ± 3.5; d = 1.17; $P < 0.01$) subjects.

The fractional extraction (%) of plasma [^{13}C]-oleate-NEFA in the muscle in the Basal period did not differ between the Lean-IS and the Obese-IR subjects (17.4 ± 6.4 vs -1.13 ± 14.7; d = 0.58; $P > 0.05$). The fractional extraction (%) of plasma [^{13}C]-oleate-NEFA in muscle in response to the insulin infusion decreased significantly from Basal and became negative (from positive) in the Lean-IS subjects (d = -3.59) indicating the release of plasma [^{13}C]-oleate-NEFAs from muscle during the insulin infusion (Figure 3B). However, the same response was not significant in the muscle of the Obese-IR subjects (d = -0.14) (Figure 3B). Moreover, the delta change from Basal

describing the fractional release (%) of plasma [^{13}C]-oleate-NEFAs from muscle in response to the insulin infusion showed a trend toward being significantly lower in the Obese-IR compared to the Lean-IS (-1.8 ± 8.7 vs -26.3 ± 9.11; *d* = 0.94; *P* = 0.07) subjects.

Blood flow

Based on previous reports (35,36), we expected that our short-term insulin infusion protocol will not modify the blood flow response in the forearm muscle. This is because insulin infusion in our studies lasted 30 minutes, a far shorter period than the time necessary (i.e., hours) to induce increase in blood flow to the muscle (35). Moreover, if insulin were to increase blood flow to muscle in the present study, this would be most evident in the Lean-IS subjects because humans with obesity and insulin resistance exhibit impaired insulin-mediated increase in blood flow in muscle (37). In order to confirm that blood flow to the forearm does not change in response to insulin infusion under the current experimental conditions, we measured branchial artery blood flow using Doppler ultrasound in the Basal period and at the end of the 30-min Insulin infusion period in our healthy, Lean-IS subjects. In these subjects, the branchial artery blood flow ($ml \cdot min^{-1}$) response to the insulin infusion did not differ from Basal (107 ± 8 vs 102 ± 12; *d* = 0.71; *P* = 0.39). Therefore, in the absence of changes in the blood flow to the forearm in response to the insulin infusion, differences in the measured A-V metabolite concentrations before and after insulin infusion across the forearm in the present study indicate differences in the uptake (i.e., if positive A-V value) or release (i.e., if negative A-V value) of metabolites across muscle.

Apolipoproteins CII and CIII

The concentration of plasma apolipoprotein-CII ($mg \cdot L^{-1}$) measured in the fasting state did not differ between the Lean-IS (31 ± 6) and Obese-IR (23 ± 3) subjects (d = 0.52; P = 0.16). However, the concentration of plasma apolipoprotein-C-III ($mg \cdot L^{-1}$) was significantly higher in the Obese-IR (36 ± 5) than the Lean-IS (26 ± 2.0) subjects (d = 0.98; P = 0.04).

Discussion

We investigated how acute increase in plasma insulin affects the handling of circulating lipids in the muscle of humans with obesity and insulin resistance versus lean, insulin-sensitive humans, as well as differences in the handling of these plasma lipids depending on their source of origin (i.e., dietary versus endogenously-produced lipids). Increased plasma insulin immediately inhibited the fractional extraction of the endogenous, but not the dietary, plasma TGs in the muscle of the lean, insulin sensitive subjects. However, similar response was not evident in the muscle of humans with obesity and insulin resistance. Failure of increased plasma insulin to suppress the extraction of TGs from endogenous sources in the skeletal muscle of humans with obesity exposes the muscle of these individuals to higher amounts of fatty acids originating from these TGs during the postprandial period, given the already elevated plasma TG concentrations from endogenous sources in humans with obesity.

Our findings show that plasma insulin plays a crucial part in acutely suppressing the fractional extraction of circulating TGs, likely by lowering the muscle intravascular LPL activity. This physiological response to increased plasma insulin lessens the amount of fatty acids released by plasma TGs in muscle, which can impact lipid accumulation in muscle. Research in rodents using a muscle-specific LPL knockout mouse model shows that lower muscle LPL activity results in lower lipid accumulation in muscle together with enhanced insulin sensitivity (38). On the other

hand, increased muscle-specific LPL expression results in increased lipid accumulation and insulin resistance in muscle (39), which are concurrent observations also in the muscle of humans with obesity (40). Although increased muscle lipid accumulation in physically active humans is not a concern because of a concurrent efficient lipid utilization in their muscle (41), increased muscle lipid accumulation in humans with obesity is causally linked to insulin resistance (42). Our study indicates that increased lipid accumulation in the muscle of humans with obesity and insulin resistance, may be due, at least in part, to plasma insulin's inability to reduce the extraction of endogenously-produced circulating TGs within the muscle of these individuals. This is likely due to the plasma insulin's inability to lower the LPL activity in the muscle vasculature of humans with obesity when plasma insulin levels rise, as happens after meal ingestion.

There is very little knowledge about how LPL is regulated in skeletal muscle *in vivo*. When assessed *in vitro*, muscle LPL activity is lower in humans with obesity and insulin resistance (43). Our findings, which demonstrate that *in vivo* the fractional extraction of circulating TGs is lower in humans with obesity and insulin resistance than in healthy controls supports this early evidence. The mechanisms that sustain lower basal-state fractional extraction of plasma TGs (i.e., LPL activity) in the muscle of humans with obesity and insulin resistance are currently unknown. Such response may result from an overall lower LPL gene expression in the muscle of humans with obesity (44), though this is not a consistent finding (13). We measured the plasma levels of APOC-III because APOC-III directly inhibits LPL from hydrolyzing TGs (45). We found the plasma concentrations of APOC-III were higher in our subjects with obesity and insulin resistance, which is consistent with previous reports (46,47). Therefore, in the non-insulin stimulated condition, lower fractional extraction of TGs from plasma in the muscle of humans with obesity and insulin resistance may be sustained by larger amounts of APOC-III in the plasma of these individuals.

Regardless of the mechanisms involved, lower fractional extraction of plasma TGs by LPL at the level of muscle in humans with obesity and insulin resistance reduces the exposure of the muscle of these individuals to lipids from circulating TGs, an effect that is expected to minimize further increase in the already elevated lipid content in the muscle of these individuals.

Expanding upon our findings from the basal, non-insulin stimulated state, our study provides novel evidence showing that the physiological role of plasma insulin is to acutely (i.e., within minutes) inhibit the function of the LPL in the muscle vasculature, as demonstrated by the decrease in the extraction of plasma TGs in the muscle of the healthy, insulin sensitive subjects. Since hyperinsulinemia associated with the postprandial state has no effect on the gene expression of LPL in muscle (13), it is expected that the increase in plasma insulin in the present study downregulates the activity, but not the content, of LPL in muscle. Indeed, hormones and dietary factors are primary regulators of the activity of the LPL (14,15). Using the particular experimental design in the present study, we were able to isolate the specific effects of plasma insulin on muscle intravascular lipolytic activity and show that the insulin resistance state is linked to an inflexibility in plasma insulin's ability to modify this lipolytic activity in muscle.

At this stage, we can only speculate about the mechanisms by which increased plasma insulin acutely reduces the rate of intravascular TG hydrolysis in the muscle of healthy humans. Although insulin can reduce the gene expression of LPL in muscle (44), it is unlikely that this mechanism is of physiological importance on upregulating the quantity and timely transport of LPL to the muscle vasculature within the 30 minutes of the insulin infusion in the present study. Indeed, the effect of plasma insulin on decreasing the mRNA expression of LPL in muscle is evident only after several hours of insulin infusion (44). On the other hand, a well-characterized effect of plasma insulin on the muscle vasculature is the stimulation of nitric oxide production in the endothelial cells (48),

and several pieces of evidence show that nitric oxide decreases the activity of LPL (49,50,51). Increased availability of nitric oxide causes nitration of LPL, which in turn reduces the *in vivo* activity of LPL (52), and it is likely a mechanism mediating the effects of increased plasma insulin in decreasing the fractional extraction of plasma TGs in the muscle of our healthy, insulin sensitive subjects. Because endothelial nitric oxide production is impaired in obesity and the insulin resistant state (53,54), this impairment would prevent any nitric oxide-mediated effects of plasma insulin on decreasing further the LPL activity in the muscle of our subjects with obesity and insulin resistance. Therefore, it is critical to investigate how nitric oxide functions as a mediator of an insulin-induced reduction in LPL activity in muscle to reduce plasma TG hydrolysis in muscle and how this process may be altered in humans with obesity and insulin resistance.

It is important to emphasize that lower fractional extraction of circulating TGs by the muscle in the Basal/non-insulin stimulated state in the subjects with obesity and insulin resistance does not imply lower exposure of muscle to fatty acids liberated by extracted plasma TGs during this period. This is because that the fractional extraction of plasma TGs in muscle must be evaluated in the context of the prevailing TG concentrations in plasma in order to assess an absolute amount of fatty acids liberated by the circulating TGs in the muscle. In this regard, compared to the lean, insulin-sensitive subjects, the plasma TG concentrations in our subjects with obesity and insulin-resistance were 2.3 times greater. Because of this, the subjects with obesity and insulin resistance will not differ in terms of the total quantity of TGs hydrolyzed in muscle when compared to the lean controls and, consequently, in the muscle's exposure to fatty acids released by these TGs in the Basal/non-insulin stimulated state. Supporting this notion, the A-V differences for plasma TGs were not different between the two groups at Basal (Table 4). On the other hand, the inhibition in the fractional extraction of plasma TGs during the insulin infusion was markedly less in the

subjects with obesity and insulin resistance (i.e., only a 1.2-fold decrease compared to a 2.5-fold decrease in the lean, insulin-sensitive subjects). This suggests that the absolute extraction of plasma TGs during the insulin infusion remained higher in the subjects with obesity and insulin resistance, while it decreased compared to the Basal/non-insulin stimulated state in the lean, insulin-sensitive subjects. Supporting this notion, the A-V difference for plasma endogenously-produced TGs tended to be significantly ($P = 0.07$) higher in the subjects with obesity and insulin resistance. Therefore, our study shows that the muscle of humans with obesity and insulin resistance is exposed to similar amounts of fatty acids liberated by the intravascular lipolysis of circulating TGs in the basal (e.g., fasted) state as that of the lean, insulin sensitive humans. However, the muscle of humans with obesity and insulin resistance is exposed to greater amounts of fatty acids liberated by the intravascular lipolysis of circulating TGs when plasma insulin increases, such as during the postprandial state.

The proportion of fatty acids taken up by the muscle from circulating TGs has been a topic of considerable debate. Original evidence suggested that 100% of the fatty acids liberated by the action of muscle LPL on plasma TGs are taken up by the muscle (9). However, other evidence indicates that the muscle releases a considerable portion of the plasma TG-liberated fatty acids in muscle back into the circulation as NEFAs (23). Our findings in healthy, insulin-sensitive humans, demonstrate that acute increase in plasma insulin results in pronounced release of NEFAs from muscle. This is supported by the NEFA A-V concentration difference across the muscle, which transitioned from a positive value in the basal state, which is consistent with prior findings using the forearm A-V model (55), to negative value during the insulin infusion. These latter findings of our investigation in the presence of increased plasma insulin corroborate prior findings showing an increase in the release of NEFA from muscle, specifically during the early postprandial period

when the plasma insulin levels spike (13). These fatty acids are likely fatty acids liberated from TGs as they pass through muscle, because muscle does not release fatty acids into the plasma from its lipid stores (56). Therefore, our research indicates that when plasma insulin levels increase in healthy humans, fatty acids liberated by the action of muscle LPL on circulating TGs are diverted away from the skeletal muscle. However, the A-V concentration difference across muscle for NEFAs derived from both endogenous and dietary sources was affected less by the increased plasma insulin in our insulin-resistant subjects. Our research, therefore, indicates that the muscle of humans with obesity and insulin resistance has greater exposure to fatty acids liberated from circulating TGs as they pass through muscle during the postprandial period.

At the basal, non-insulin stimulated, state the fractional extraction of plasma NEFAs in muscle was not different between subjects with obesity and lean controls, which supports previous observations (7). Our findings showing that increased plasma insulin suppresses the fractional extraction of plasma NEFAs in skeletal muscle parallel those of previous research showing that insulin decreases the fractional extraction of plasma NEFAs in heart muscle (57). The effects of plasma insulin on the fractional extraction plasma NEFAs in muscle are likely not related to direct effects of plasma insulin in skeletal muscle. This is because experiments employing the perfused rodent muscle model show that NEFA uptake in muscle is not insulin dependent (58). The inability of plasma insulin to suppress the rate of appearance of fatty acids from extramuscular (i.e., adipose) tissues in the insulin resistant subjects postprandially (59) is rather a key mechanism for the lower suppression of the extraction of plasma NEFAs in the muscle in our subjects with obesity and insulin resistance during the insulin infusion. This led to the observed larger concentrations of NEFAs in arterial plasma in our subjects with obesity and insulin resistance when compared to the lean, insulin-sensitive subjects during the insulin infusion, and which likely mediated greater

fractional extraction of plasma NEFAs in muscle in the subjects with obesity and insulin resistance during insulin infusion. Additionally, the higher number of CD36 fatty acid transporters reported to exist in the sarcolemma of muscle cells of subjects with obesity (60) may be a contributing factor to the larger fractional extraction of plasma NEFAs in the presence of increased insulin in our subjects with obesity. Specifically, increased content of muscle CD36 fatty acid transporters, which have key role in transporting fatty acids into muscle (61), in humans with obesity and insulin resistance may have sustained increased extraction of plasma NEFAs by the muscle in these subjects during the insulin infusion and regardless of the concentrations of NEFAs in plasma. Therefore, higher fractional extraction of plasma NEFAs by the muscle of humans with obesity and insulin resistance postprandially when compared to lean, insulin sensitive humans contributes to greater lipid accumulation in the muscle of these individuals. This is analogous to the circumstance in which experimental increase in the plasma NEFA concentrations causes lipid accumulation in skeletal muscle (62).

A potential limitation of the current investigation could be considered the lack of net flux values for TGs and NEFAs across muscle. Previous reports have calculated net flux (i.e., net uptake or release) of metabolites across muscle, which is accomplished by multiplying the muscle A-V difference in metabolite concentrations by the limb blood flow (10,13). However, forearm blood flow estimations, which are obtained using strain gauge plethysmography, do not describe specifically blood flow to the muscle because blood flow to the forearm is a weighted average of the flow of blood to both muscle and fat tissue. Moreover, such measurements are highly variable and less reliable in humans with increased forearm adiposity (63), as is the case in our study (Table 1). Consequently, in the present study we contrasted only parameters that are independent of blood flow measurements, with the fractional extraction of metabolites serving as the main end-point.

Given the absence of differences in blood flow in muscle between the insulin and noninsulin-stimulated states, the provided data describing A-V concentration differences in metabolites across muscle strengthen our conclusions as they relate to the kinetics of metabolites across muscle. Therefore, using a carefully-designed mechanistic experiments we describe how plasma insulin regulates the metabolism of circulating TGs and NEFAs across muscle in the postprandial state in healthy humans and those with obesity and insulin resistance. Our studies shed light into the complex biology that accounts for increased extraction (12) and greater accumulation of lipids from dietary sources (64) postprandially in the muscle of humans with obesity and insulin resistance.

Conclusions

We demonstrate for the first time a direct physiological role of plasma insulin in partitioning plasma lipids away from skeletal muscle by inhibiting the fractional extraction of plasma triglycerides from endogenous (but not from dietary) sources and by reducing the fractional extraction of circulating NEFAs in muscle. Importantly, we show that these plasma insulin-mediated responses in muscle are impaired in humans with obesity and insulin resistance. Specifically, we show that the insulin resistant state, which is already recognized to impair glucose metabolism in muscle (65), also has adverse impact on the metabolism of circulating lipids as they travel through the muscle. This impairment in humans with obesity and insulin resistance is documented as inability to reduce the intravascular fractional extraction of plasma lipids in muscle in response to the postprandial increase in plasma insulin.

Acknowledgments

We gratefully acknowledge the assistance of the subject recruitment team and the nursing staff in the Clinical Studies Infusion Unit at Mayo Clinic in Scottsdale, Arizona for their support in conducting the studies. We also thank the subjects for their participation and commitment to the study procedures.

Funding

The study was supported by American Diabetes Association grant # 7-12-CT-40 and the Mayo Clinic Metabolomics Resource Core through grant number U24DK100469 from the NIH/NIDDK and the Mayo Clinic CTSA grant UL1TR000135 from the National Center for Advancing Translational Sciences.

Disclosures

The authors have nothing to disclose.

Data Availability

Original data generated and analyzed during this study are included in this published article.

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Figure Legends

Figure 1. Depiction of the experimental protocol employed to determine the postprandial extraction of plasma triglycerides and non-esterified fatty acids prior to (Basal) and following (Insulin) insulin infusion. [1,1,1-$^{13}C_3$]triolein-enriched whipping cream was ingested at the time points indicated in the form of small boluses to induce steady-state in the concentrations of ingested and overall plasma triglycerides. Arterio-venous (A-V) blood samples were collected from a radial artery and deep vein draining forearm muscle.

Figure 2. Forearm muscle fractional extraction (**A**) and concentrations (**B**) of plasma glucose prior to (Basal) and following insulin infusion (Insulin) in lean, insulin sensitive humans (Lean – IS) and humans with obesity and insulin resistance (Obese - IR). Data are mean ± SEM, along with individual data points for plasma glucose fractional extraction. Data were analyzed by a two-way ANOVA followed by pairwise comparisons using the Bonferroni multiple comparisons test.

Figure 3. Forearm muscle fractional extraction of plasma dietary (i.e., [^{13}C]-oleate-TG) (**A**) and endogenously-produced (i.e., Unlabeled-oleate-TG) (**B**) triglyceride (TG) prior to (Basal) and following insulin infusion (Insulin) in lean, insulin sensitive humans (Lean – IS) and humans with obesity and insulin resistance (Obese - IR). Data are mean ± SEM, along with individual data points. Data were analyzed by a two-way ANOVA followed by pairwise comparisons using the Bonferroni multiple comparisons test.

Figure 4. Forearm muscle fractional extraction of plasma dietary (i.e., [^{13}C]-oleate-NEFA) (**A**) and endogenous (i.e., Unlabeled-oleate-NEFA) (**B**) non-esterified fatty acids (NEFA) prior to (Basal) and following insulin infusion (Insulin) in lean, insulin sensitive humans (Lean – IS) and humans with obesity and insulin resistance (Obese - IR). Data are mean ± SEM, along with individual data points. Data were analyzed by a two-way ANOVA followed by pairwise comparisons using the Bonferroni multiple comparisons test.

Table 1. Subject characteristics

	Lean - IS	Obese - IR
n (F/M)	9 (2/7)	8 (3/5)
Age (years)	24.6 ± 1.6	27.3 ± 3.0
Weight (kg)	70.6 ± 3.4	107.7 ± 7.2***
BMI (kg/m^2)	22.4 ± 0.8	35.0 ± 2.2***
Lean body mass (kg)	55.7 ± 2.3	65.0 ± 4.7
Forearm lean mass (kg)	0.99 ± 0.07	0.99 ± 0.14
Forearm fat mass (kg)	0.20 ± 0.03	0.56 ± 0.08***
Forearm fat mass (%)	15.7 ± 2.5	35.3 ± 5.5**
Body fat mass (%)	17.5 ± 1.8	36.6 ± 3.6***
Android/Gynoid Fat	0.29 ± 0.03	0.62 ± 0.07***
Visceral Fat Mass (g)	287 ± 68	714 ± 135*
Fasting plasma glucose (mmol·L^{-1})	4.6 ± 0.1	5.0 ± 0.2*
Fasting plasma insulin (µIU·ml^{-1})	2.9 ± 0.3	11.7 ± 1.4***
HOMA-IR	0.6 ± 0.1	2.6 ± 0.3***
Matsuda-ISI	11.5 ± 1.2	3.1 ± 0.3***
HbA1c (mmol·mol^{-1})	33.5 ± 1.6	34.0 ± 1.4
HbA1c (%)	5.2 ± 0.1	5.3 ± 0.1
TSH (mIU·l^{-1})	1.3 ± 0.3	1.5 ± 0.3
Plasma triglycerides (mmol·L^{-1})	0.8 ± 0.1	1.6 ± 0.2**
Total plasma cholesterol (mmol·L^{-1})	3.8 ± 0.2	4.1 ± 0.2
Plasma HDL-Cholesterol (mmol·L^{-}	1.4 ± 0.1	1.0 ± 0.1***
Plasma LDL-Cholesterol (mmol·L^{-1})	2.0 ± 0.3	2.4 ± 0.2

Values are mean ± SEM. Lean-IS, lean, insulin sensitive subjects; Obese-IR, subjects with obesity and insulin resistance; BMI, body mass index; Android/Gynoid fat, ratio of android fat mass to gynoid fat mass; HOMA-IR, homeostatic model assessment of insulin-resistance; Matsuda-ISI, Matsuda Insulin Sensitivity Index; HbA1c, glycated hemoglobin; TSH, thyroid-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, significantly different from Lean-IS.

Table 2. Concentration of insulin and metabolites in arterial plasma during fasting, the basal period and insulin infusion

	Fasting	Basal	Insulin	ANOVA (*P Value*) Interaction	Group	Time
Insulin (μIU·ml^{-1})				*0.37*	*< 0.001*	*< 0.001*
Lean-IS	4.0 ± 0.6	4.4 ± 0.7	53.3 ± 3.3			
Obese-IR	15.2 ± 1.2	16.8 ± 3.1	74.9 ± 9.1			
Glucose (mmol·L^{-1})				*0.42*	*0.45*	*0.82*
Lean-IS	4.4 ± 0.1	4.5 ± 0.1	4.4 ± 0.1			
Obese-IR	4.6 ± 0.2	4.6 ± 0.2	4.6 ± 0.1			
Total-TG (μmol·L^{-1})				*0.76*	*0.02*	*0.31*
Lean-IS	585 ± 68	642 ± 91	589 ± 95			
Obese-IR	1297± 280	1437 ± 338	1384 ± 329			
Unlabeled-Oleate-TG (μmol·L^{-1})				*0.78*	*0.05*	*0.07*
Lean-IS	N/A	218 ± 35	198 ± 36			
Obese-IR	N/A	493 ± 135	479 ± 132			
[^{13}C]-oleate-TG (μmol·L^{-1})				*0.24*	*0.21*	*0.16*
Lean-IS	N/A	1.5 ± 0.4	1.6 ± 0.3			
Obese-IR	N/A	2.8 ± 1.1	3.5 ± 1.5			
Total-NEFA (μmol·L^{-1})				*0.17*	*0.07*	*<0.001*
Lean-IS	418 ± 72	430 ± 36	101 ± 12			
Obese-IR	444 ± 53	496 ± 48	281 ± 38			
Unlabeled-oleate-NEFA				*0.10*	*0.02*	*<0.001*
Lean-IS	N/A	159 ± 14	30 ± 3			
Obese-IR	N/A	174 ± 20	89 ± 13			
[^{13}C]-oleate-NEFA (μmol·L^{-1})				*0.01*	*0.55*	*0.42*
Lean-IS	N/A	0.96 ± 0.17	0.52 ± 0.07			
Obese-IR	N/A	0.51 ± 0.08	0.76 ± 0.22			

Values are mean ± SEM; Lean-IS, lean, insulin sensitive subjects; Obese-IR, subjects with obesity and insulin resistance; TG, triglycerides; NEFA, non-esterified fatty acids.

Table 3. Isotopic enrichment of plasma triglycerides and non-esterified fatty acids with [^{13}C]-oleate in arterial and venous plasma in the basal period and during insulin infusion

	Basal	Insulin	ANOVA (*P value*) Interaction	Group	Time
TG (TTR) - Arterial			*0.55*	*0.32*	*0.02*
Lean-IS	0.007 ± 0.001	0.008 ± 0.001			
Obese-IR	0.005 ± 0.001	0.007 ± 0.002			
TG (TTR) - Venous			*0.78*	*0.69*	*<0.01*
Lean-IS	0.005 ± 0.001	0.006 ± 0.002			
Obese-IR	0.004 ± 0.001	0.006 ± 0.001			
NEFAs (TTR) - Arterial			*<0.01*	*<0.01*	*<0.001*
Lean-IS	0.006 ± 0.001	0.017 ± 0.001			
Obese-IR	0.003 ± 0.001	0.008 ± 0.002			
NEFA (TTR) - Venous			*0.06*	*0.06*	*<0.001*
Lean-IS	0.005 ± 0.001	0.012 ± 0.001			
Obese-IR	0.003 ± 0.001	0.007 ± 0.002			

Values are tracer-to-tracee ratio (TTR) mean ± SEM; Lean-IS, lean, insulin sensitive subjects; Obese-IR, subjects with obesity and insulin resistance; TG, triglycerides; NEFA, non-esterified fatty acids.

Table 4. Arterio-venous differences of metabolites in the basal period and during insulin infusion

	Basal	Insulin	ANOVA (*P value*) Interaction	Group	Time
Glucose (mmol·L^{-1})			*0.03*	*0.01*	*<0.001*
Lean-IS	0.23 ± 0.04	0.81 ± 0.13			
Obese-IR	0.12 ± 0.02	0.35 ± 0.09			
Total-TG (µmol·L^{-1})			*0.59*	*0.46*	*0.03*
Lean-IS	55 ± 15	18 ± 6			
Obese-IR	63 ± 27	40 ± 14			
Unlabeled-oleate-TG (µmol·L^{-1})			*0.18*	*0.20*	*0.09*
Lean-IS	21 ± 7	6 ± 3			
Obese-IR	26 ± 11	24 ± 7			
^{13}C-Oleate-TG (µmol·L^{-1})			*0.60*	*0.62*	*0.12*
Lean-IS	0.58 ± 0.23	0.29 ± 0.16			
Obese-IR	0.96 ± 0.61	0.40 ± 0.48			
Total-NEFA (µmol·L^{-1})			*0.04*	*0.50*	*<0.001*
Lean-IS	16.5 ± 17.8	-45.5 ± 14.6			
Obese-IR	14.8 ± 17.7	-13.8 ± 14.4			
Unlabeled-oleate-NEFA (µmol·L^{-1})			*0.06*	*0.53*	*<0.001*
Lean-IS	8.7 ± 8.2	-16.4 ± 6.1			
Obese-IR	8.1 ± 6.4	-4.3 ± 5.3			
^{13}C-Oleate-NEFA (µmol·L^{-1})			*0.04*	*0.53*	*0.05*
Lean-IS	0.18 ± 0.07	-0.06 ± 0.06			
Obese-IR	0.01 ± 0.06	0.01 ± 0.06			

Values are mean ± SEM; Lean-IS, lean, insulin sensitive subjects; Obese-IR, subjects with obesity and insulin resistance; TG, triglycerides; NEFA, non-esterified fatty acids.

Figure 1

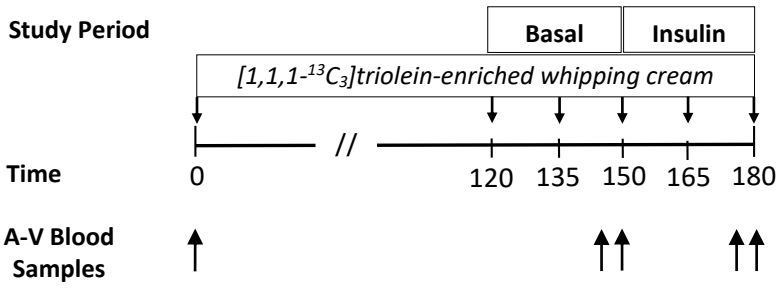

Study Period
Basal
Insulin
[1,1,1-13C3]triolein-enriched whipping cream
//
Time
0
120
135
150
165
180
A-V Blood Samples

Figure 2

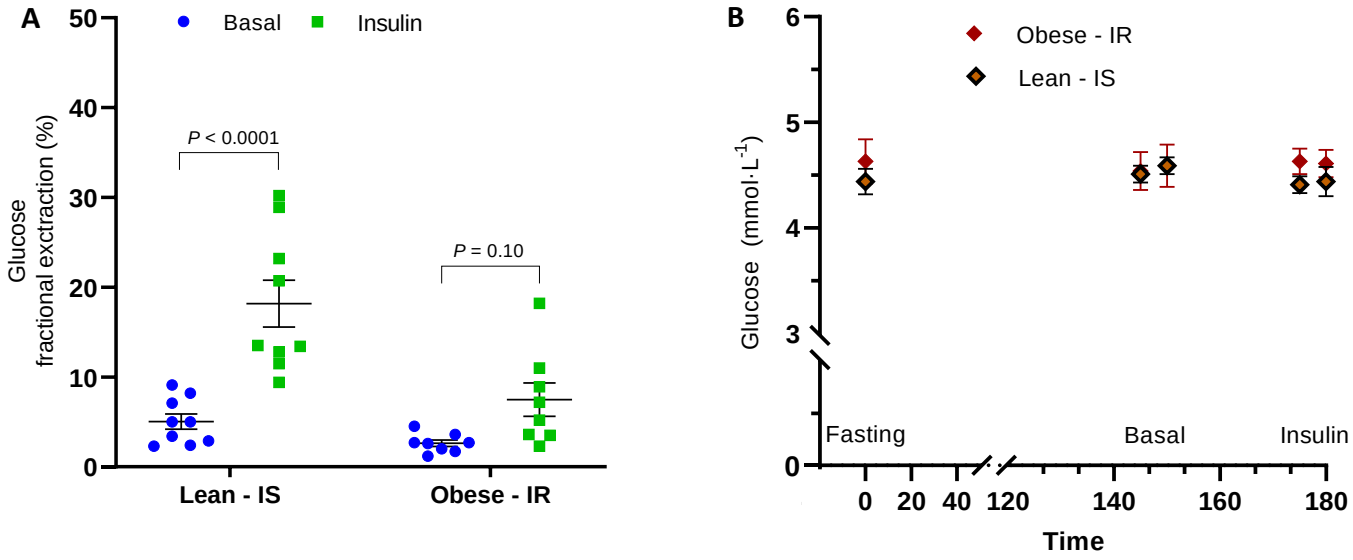

A
Basal
Insulin
Glucose
fractional exctraction (%)
50
40
30
20
10
0
P < 0.0001
P = 0.10
Lean - IS
Obese - IR
B
Obese - IR
Lean - IS
Glucose (mmol·L-1)
6
5
4
3
0
Fasting
Basal
Insulin
0
20
40
120
140
160
180
Time

Figure 3

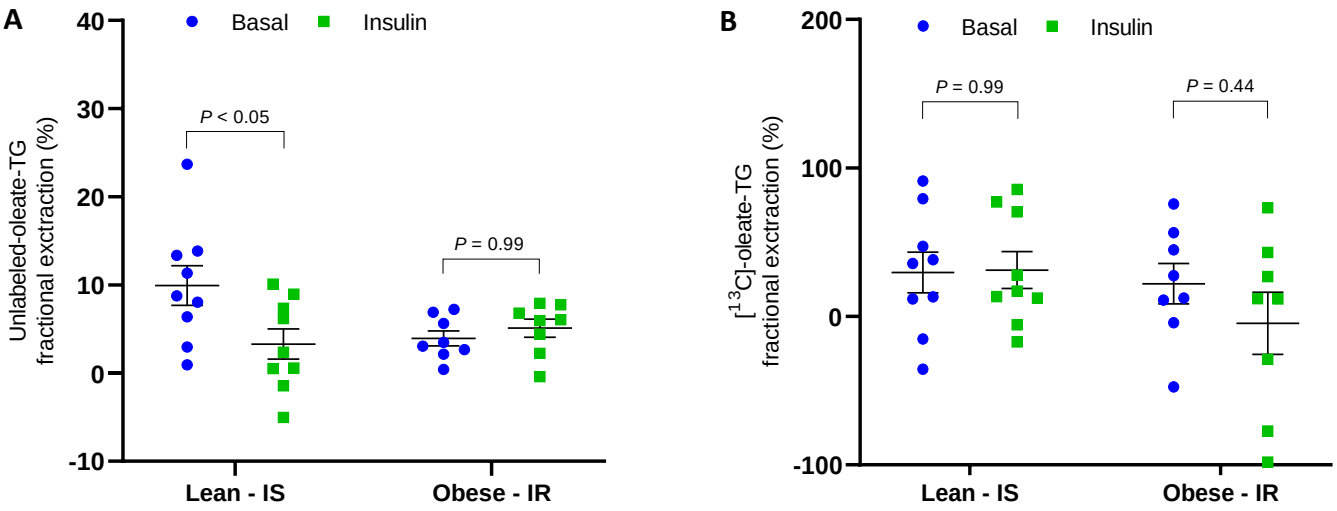
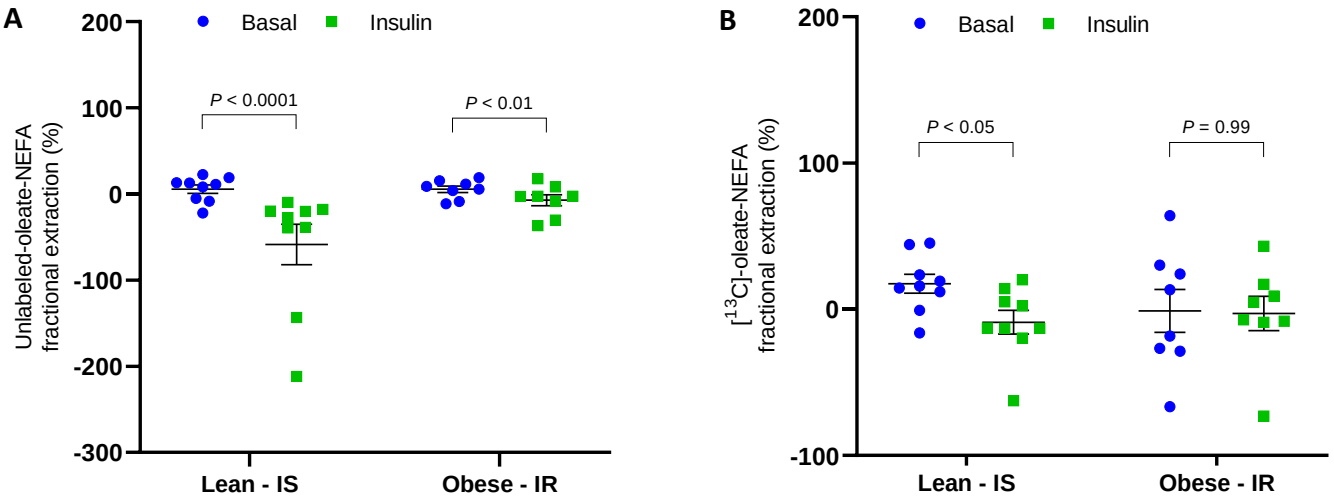

A
Basal
Insulin
Unlabeled-oleate-TG fractional exctraction (%)
40
30
20
10
0
-10
P < 0.05
P = 0.99
Lean - IS
Obese - IR
B
Basal
Insulin
[13C]-oleate-TG fractional exctraction (%)
200
100
0
-100
P = 0.99
P = 0.44
Lean - IS
Obese - IR

Figure 4


A
Basal
Insulin
Unlabeled-oleate-NEFA fractional extraction (%)
200
100
0
-100
-200
-300
P < 0.0001
P < 0.01
Lean - IS
Obese - IR
B
Basal
Insulin
[13C]-oleate-NEFA fractional extraction (%)
200
100
0
-100
P < 0.05
P = 0.99
Lean - IS
Obese - IR