Differential regulation of lipid and protein metabolism in obese vs. lean subjects before and after a 72-h fast

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Bak AM, Møller AB, Vendelbo MH, Nielsen TS, Viggers R, Rungby J, Pedersen SB, Jørgensen JO, Jessen N, Møller N. Differential regulation of lipid and protein metabolism in obese vs. lean subjects before and after a 72-h fast. Am J Physiol Endocrinol Metab 311: E224–E235, 2016. First published May 31, 2016; doi:10.1152/ajpendo.00464.2015.—Increased availability of lipids may conserve muscle protein during catabolic stress. Our study was designed to define 1) intracellular mechanisms leading to increased lipolysis and 2) whether this scenario is associated with decreased amino acid and urea fluxes, and decreased muscle amino acid release in obese subjects under basal and fasting conditions. We therefore studied nine lean and nine obese subjects twice, after 12 and 72 h of fasting, using measurements of mRNA and protein expression and phosphorylation of lipolytic and protein metabolic signaling molecules in fat and muscle together with whole body and forearm tracer techniques. Obese subjects displayed increased whole body lipolysis, decreased urea production rates, and decreased forearm muscle protein breakdown per 100 ml of forearm tissue, differences that persisted after 72 h of fasting. Lipolysis per fat mass unit was reduced in obese subjects and, correspondingly, adipose tissue hormone-sensitive lipase (HSL) phosphorylation and mRNA and protein levels of the adipose triglyceride lipase (ATGL) coactivator CGI58 were decreased. Fasting resulted in higher HSL phosphorylations and lower protein levels of the ATGL inhibitor G0S2. Muscle protein expressions of mammalian target of rapamycin (mTOR) and 4EBP1 were lower in obese subjects, and MuRF1 mRNA was higher with fasting in lean but not obese subjects. Phosphorylation and signaling of mTOR decreased with fasting in both groups, whereas ULK1 protein and mRNA levels increased. In summary, obese subjects exhibit increased lipolysis due to a large fat mass with blunted lipolytic signaling, together with decreased urea and amino acid fluxes both in the basal and 72-h fasted state; this is compatible with preservation of muscle and whole body protein.

obesity; fasting; protein breakdown; lipolysis; skeletal muscle; subcutaneous adipose tissue; human

FASTING AND OBESITY ARE BOTH associated with increased levels of free fatty acids (FFAs) In the fasting state, FFA mobilization is promoted by the combined effects of reduced plasma insulin and increased levels of catecholamines, growth hormone, and cortisol (41) and, remarkably, decreased lipolytic effect of catecholamines in adipose tissue (AT) has consistently been demonstrated in obese subjects (12, 31, 32). In adipocytes, lipolysis is controlled by the sequential action of adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase that hydrolyze triglycerides to FFA and glycerol (25). It has previously been shown that ATGL protein levels increase and that the ATGL inhibitor G0/G1 switch gene 2 (G0S2) protein and mRNA levels decrease during fasting in humans (26). A number of studies have shown that FFA and ketone bodies are decisive for protein conservation, in particular during fasting (6, 22, 27), but the underlying mechanisms remain elusive.

The metabolic effects of fasting in obese subjects have been studied extensively, but the effects of obesity on amino acid metabolism remain unclear, and data from studies measuring protein kinetics are conflicting. One study (11) reported that obesity was associated with increased postabsorptive proteolysis and impaired antiproteolytic actions of insulin, and another study (15) found normal basal endogenous leucine fluxes and oxidation rates but impaired anabolic responses to insulin. A third study (21) showed increased rates of postabsorptive protein synthesis in obese subjects. All studies used leucine tracers and reported values per lean body mass (LBM) unit, thus dismissing any contribution from AT. A later study (29) scrutinizing this issue reported that although whole body protein breakdown measured with leucine was similar after a 22-h fast in obese and lean women when calculated per LBM, muscle forearm leucine release per 100 ml of forearm tissue was dramatically (>50%) decreased in obese subjects and that AT accounted for up to 10% of whole body protein breakdown in both lean and obese subjects. It has been estimated that skeletal muscle contains between 30 and 45% of total body protein and contributes between 20 and 35% of whole body protein turnover (1). With fasting, it has been reported that up to 2 wk of starvation did not increase the leucine rate of appearance/protein breakdown in obese subjects, whereas 60 h of fasting resulted in higher leucine rates of appearance and oxidation rates in lean subjects (37). The regulation of whole body and muscle protein metabolism is a matter of complexity. Maintenance of protein and muscle mass depends on the dynamic equilibrium between protein synthesis and protein
breakdown, which can be assessed by measuring the rates of appearance (breakdown) and disappearance (synthesis) of a labeled amino acid tracer. Regional leg or forearm muscle protein metabolism can be assessed by combining infusion of labeled amino acid tracers with measurements of local arterio-venous balances and blood flows, but the effects of fasting on regional muscle protein metabolism in obese subjects are uncertain. Recent research has highlighted the pivotal role of intracellular signal cascades in regulating this balance. The mammalian target of rapamycin (mTOR), which is a highly preserved serine/threonine kinase, has emerged as a master regulator of protein synthesis signaling in muscle tissue; in addition, mTOR suppresses autophagy and protein breakdown by modulating Unc-51-like kinase (ULK1) phosphorylation (33). Autophagy is an evolutionary conserved process of degradation of intracellular proteins, which is regulated and initiated by activation of ULK1 (33).

Throughout evolution fasting has been inevitable, and it is likely that natural selection has favored survival of subjects with high metabolic capacity to cope with long-term fasting (23). During fasting, high levels of FFAs restrict protein breakdown and urea formation (27), and AT serves as an energy reservoir, which may prolong survival time and conserve lean tissue to maintain vital body functions. Obese subjects have higher levels of FFAs and display $>50\%$ reduced protein and urinary nitrogen loss during starvation and, in some studies, display evidence of decreased protein oxidation during the first days of fasting (9). It is uncertain which metabolic and signaling mechanisms initiate and sustain these events in AT and muscle.

On the basis of these considerations, our study was designed to test whether obese subjects display increased lipolysis and decreased muscle amino acid release, and whether these adaptations persist during prolonged fasting. In addition, we hypothesized that such adaptations could be mediated by modifications in adipose tissue ATGL- and HSL-related signaling and alterations in muscle mTOR and ULK1-mediated signaling to protein synthesis and autophagy. To allow direct assessment of muscle protein kinetics we employed a combination of intracellular signal cascades in regulating this balance. The rate of phenylalanine conversion by hydroxylation to tyrosine ($\frac{Q_{phe}}{Q_{tyr}}$) was calculated as $Q_{phe} = Q_{tyr} \times \left(\frac{15N-TyrEi}{15N-PheEi} \times \frac{Q_{phe}}{I_{phe} + Q_{phe}}\right)$, where $15N-TyrEi$ and $15N-PheEi$ are the isotopic enrichments of the modification in respective tracers in plasma, and $I_{phe}$ is the infusion rate of $[15N]$phenylalanine ($\mu$mol/$(kg \cdot h)$), $E_i$ and $E_p$ are enrichment of the tracer infused, and plasma enrichment of the tracer at isotopic plateau, respectively (10, 36).

The rate of phenylalanine conversion by hydroxylation to tyrosine ($Q_{phe}$) was calculated as $Q_{phe} = Q_{tyr} \times \left(\frac{15N-TyrEi}{15N-PheEi} \times \frac{Q_{phe}}{I_{phe} + Q_{phe}}\right)$, where $15N-TyrEi$ and $15N-PheEi$ are the isotopic enrichments of the modification in respective tracers in plasma, and $I_{phe}$ is the infusion rate of $[15N]$phenylalanine ($\mu$mol/$(kg \cdot h)$) (18).

Phenylalanine balance was calculated as follows using Fick’s principle: phenylalanine balance = ($P_{phe} - P_{phe}$) $\times$ F, in which $P_{phe}$ and $P_{phe}$ are venous and arterial phenylalanine concentrations, respectively, and F is blood flow in the forearm. Regional phenylalanine kinetics were calculated using the equations described by Nair et al. (20). The forearm protein breakdown represented by phenylalanine was calculated as $Q_{phe} = Q_{tyr} \times \left(\frac{15N-TyrEi}{15N-PheEi} \times \frac{Q_{phe}}{I_{phe} + Q_{phe}}\right)$, where $15N-TyrEi$ and $15N-PheEi$ are the isotopic enrichments of the modification in respective tracers in plasma, and $I_{phe}$ is the infusion rate of $[15N]$phenylalanine ($\mu$mol/$(kg \cdot h)$) (18).

Physical Activity Levels

Average duration and type of daily physical activity were recorded by the subjects on a questionnaire, resulting in one total score for each individual.

Body Composition

Dual-energy X-ray absorptiometry (DEXA) scans were performed once at the initial medical examination (QDR-2000; Hologic, Marlborough, MA) to determine LBM, fat mass, and fat percentages. Furthermore, regions of interest were set on the forearms to estimate fat and lean tissue forearm content.

Amino Acid Tracers

Muscle amino acid balances were assessed by the forearm method (17). Total forearm blood flow was measured by venous occlusion plethysmography (40). $\frac{[15N]}{[2H4]}$phenylalanine, $\frac{[15N]}{[H3]}$tyrosine, $\frac{[15N]}{[13C]}$urate (Cambridge Isotope Laboratories, Andover, MA) were infused as previously described (38).

Whole body phenylalanine ($Q_{phe}$), tyrosine ($Q_{tyr}$), and urea fluxes ($Q_{urea}$) were calculated as $Q = I \times [(E/E_i) - 1]$, in which i is the rate of tracer infusion $[\mu$mol/$(kg \cdot h)]$, $E_i$ and $E_p$ are enrichment of the tracer infused, and plasma enrichment of the tracer at isotopic plateau, respectively (10, 36).

Ethical Approval

The study was approved by the Central Denmark Region Scientific Ethics Committee (approval M-2010-0182) and was performed in compliance with the Declaration of Helsinki II. Written informed consent was obtained from all subjects.

Study Subjects

A total of 18 healthy young men between 20 and 35 yr of age participated, nine were lean [body mass index (BMI) 19–23] and nine were obese (BMI 32–40) according to the World Health Organization definition of obesity (BMI >30). One to 4 wk before the study each participant completed a medical examination, including routine blood chemistry to exclude undiagnosed illness. All subjects had low levels of physical activity, were nonsmokers, and used no prescription medications. Three subjects in the lean group dropped out during the study because of lack of compliance and were replaced with new study subjects.

Study Protocol

In a randomized crossover design, subjects were examined on two occasions separated by a minimum of 21 days: 1) in the postabsorptive basal state after an overnight fast of 12 h, and 2) after 72 h of fasting.

Exercise and alcohol intake 48 h before each fasting period were not allowed, and subjects were instructed to continue their usual diets. During the fasting periods subjects were allowed to drink water and perform normal activities, excluding exercise. Daily blood samples were taken (glucose, insulin, FFA) to ensure total compliance with the fast. At 7:00 A.M. on the study days the subjects were placed in a quiet, thermoneutral room and were studied for 6 h from 8:00 A.M. ($t = 0$) to 2:00 P.M. while remaining in bed. After a 4-h basal period ($t = 0 – 240$) a hyperinsulenic euglycemic clamp was performed ($t = 240 – 360$). Data from the clamp period will be published separately.

**Palmitate Tracer**

Systemic palmitate flux was calculated using the isotope dilution technique with a constant infusion of albumin-bound [9,10-3H]palmitate (0.3 μCi/min) (PerkinElmer, Mechelen, Belgium/Department of Clinical Physiology and Nuclear Medicine, Aarhus University Hospital, Denmark) from t = 180–240. Blood samples for measurements were drawn before the infusion and in triplicate during the last 30 min of the infusion period. Plasma palmitate concentration and specific activity (SA) were measured by HPLC using [3H]palmitate as an internal standard. Palmitate flux (μmol/min) was calculated as [9,10-3H]palmitate infusion rate (disintegrations/min) divided by the steady-state palmitate SA (disintegrations·min⁻¹·μmol⁻¹) (16, 24).

**Biopsies**

Muscle and AT biopsies were obtained under sterile conditions using local anesthesia (Lidokain; Amgros, Copenhagen, Denmark) at t = 60 (basal period) and 270 min (clamp period). Subcutaneous AT from the abdomen (~5 cm distal to the umbilicus) was obtained by biopsy and fixed in 10% formaldehyde for 2 h with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology). Proteins were visualized by chemiluminescence (Clarity Western ECL Substrate; Bio-Rad) and quantified with the ChemiDoc MP imaging system (Bio-Rad). Protein Plus Precision All Blue Standards (Bio-Rad) were used as markers of molecular weight. Membranes were initially incubated with phosphospecific antibodies. Subsequently, the antibodies were stripped off using a buffer containing 6 M guanidine hydrochloride, 0.2% NP-40, 10 mM dithiotheritol, and 20 mM Tris-HCl (pH 7.5), and the membranes were reblocked and reincubated in antibodies against total protein. Antibodies (numbers in parentheses indicate catalog numbers) against mTOR (2972), 4EBP1 (9644P) ULK1 (4773), LC3B (3868), and phospho-specific antibodies against mTOR p-Ser2448 (2971), nonphospho-4EBP1 p-Thr37/46 (2855), and ULK1 p-Ser757 (6888) were purchased from Cell Signaling Technology (Beverly, CA). Antibody against SQSTM1/p62 (ab56416) was purchased from Abcam (Cambridge, UK).

**ADIPOSE TISSUE.** Approximately 100 mg of subcutaneous AT was homogenized in HEPES buffer (50 mM HEPES, 20 mM NaF, 2 mM Na3VO4, 5 mM EDTA, 5% SDS, HALT, 5 mM NAM) and 10 μM TSNA on a Precellys 24 (Bertin Technologies, Montigny-le-Bretonneux, France). Samples were thermomixed at 37°C and swirled for 1 h, followed by centrifugation at 14,000 g for 20 min at room temperature. The homogenate was carefully separated from the lipid layer by a thin pipette and centrifuged again to homogenize the samples more even further. This procedure was repeated up to three times until the homogenate was completely clear. The homogenate was stored at −80°C until further analysis. The homogenate was mixed 1:1 with 2× Lammeli buffer and heated at 95°C for 5 min. Western blotting was performed using the same protocol as described for muscle analysis. Antibodies against HSL (4107), ATGL (2138), and phospho-specific antibodies against HSL p-Ser554 (4139), HSL p-Ser555 (4137), and HSL p-Ser650 (4126) were purchased from Cell Signaling Technology. Antibodies against G0S2 (80353) and CGI58 (80365) were purchased from Abcam (Cambridge, UK). Antibody against CIDEA (NB100-94219) was purchased from Novus Biologicals (Littletown, CO). Antibody against CIDEC (H00063924-M07) was purchased from Abnova (Taipei City, Taiwan), and antibody against PLIN1 (PA1-1052) was purchased from Thermo Scientific (Rockford, IL).

Analysis of protein expressions in AT (ATGL, G0S2, CGI58, CIDEA, CIDE, and PLIN1) from the basal and clamp periods revealed no clamp-induced changes, and the data are not presented. The basal and clamp results. Phosphorylation levels of HSL in AT were significantly affected by insulin stimulation during the clamp; therefore, only the basal HSL data are presented.

**Extraction of RNA and quantitative PCR for mRNA analysis.** RNA was extracted using TRIzol (Gibco BRL, Life Technologies, Roskilde, Denmark) and homogenized with one tungsten bead (Qiagen) using a mixer mill. RNA was quantified by measuring absorbance at 260 and 280 nm with a ratio between RNA and protein ≥1.9 using a NanoDrop 8000 Spectrophotometer (Thermo Scientific Pierce, Waltham, MA). Integrity of the RNA was checked by visual inspection of the two ribosomal RNAs on an agarose gel. Complementary DNA was synthesized using a Verso cDNA kit (Ab-1453; Thermofischer Scientific) with random hexamer primers. The PCR reactions were performed in duplicate using a KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Woburn, MA) in a LightCycler 480 (Roche Applied Science) using the following protocol: one step at 95°C for 3 min, then 95°C for 10 s, 60°C for 20 s, 72°C for 10 s, and finally, a melting curve analysis was performed. The increase in fluorescence was measured in real time during the extension step. The relative gene expression was estimated using the default “Advanced Relative Quantification” mode of the software version LCS 480 1.5.1.62 (Roche Applied Science), and specificity of the amplification was checked by a melting temperature analysis.

The following primer pairs were designed using QuantPrime (2): ATGL, ACCTCAATGACTTGGCACC and CAACGGACCCAGGAGCCAGATG.
and AGCACACGGCGGAGAGG, length = 137 bp; CGL58, TGT-CAGCGCGCTTCCGAGATAAG and ACCAGTTCACACCTGAC-CTCCT, length = 113 bp; CIDEA, CGGCTGCCCTTACGAGTA and AGATGAGAACGTGTCCCACT, length = 143 bp; CIDEF, CATTGCCTGCTTACGAGTA and GAGGTGGGCAAGCATACGTG, length = 154 bp; MuRF1, ACCGTGTGAGCCATCATCAC and ACCCTGGGACTGTTCCTTGC, length = 70 bp; ULK1, AAGGTCTAGTTCCTCCGCAAGG and CGACTCTCATA-TGGTGTTC, length = 94 bp; and atrogin, AAGAGGCGCATG-GATATGGAC and TCCAGAATGGCAGTGGAGAAGTCC, length = 70 bp. The housekeeping gene, β2 microglobulin, was amplified using this primer pair: GAGGCTATCCGCGACTGCTC and ATGGCGGATGTAACCAC, length = 111 bp. Expression level of this housekeeping gene was similar between all groups and interventions in skeletal muscle tissue. However, in AT, this housekeeping gene was similar between all groups and interventions in both groups, most markedly so in lean subjects.cases. When interactions between BMI and fasting state or main effects of BMI or fasting were found, linear pairwise comparisons were performed post hoc to compare differences within and between individual conditions. The level of significance was set at \( P < 0.05 \). Statistical analyses were performed using Stata 13.1 (StataCorp, College Station, TX). Graphs were designed in SigmaPlot 11.0 (Systat Software, San Jose, CA).

RESULTS

Specific activity of palmitate and isotopic enrichments of urea, phenylalanine, and tyrosine all reached steady state (assessed based on the observation that when specific activity/isotopic enrichment values of the tracers were plotted against time the ensuing slopes were not different from zero; data not shown).

Baseline characteristics are presented in Table 1.

| Hormones and metabolites. Serum-insulin (71 ± 10 vs. 37 ± 7 pM, \( P = 0.002 \)) and C-peptide levels were significantly higher in obese than lean subjects and decreased during prolonged fasting (Table 2). Glucagon levels increased significantly with 72 h of fasting in both groups. FFA levels were ~40% higher in obese than lean subjects after 12 h of fasting (0.55 ± 0.03 vs. 0.39 ± 0.06 mM, \( P = 0.016 \)) and increased significantly during prolonged fasting in both groups, the increase being more prominent in lean subjects. In addition, prolonged fasting caused a reduction in glucose levels in both groups, most markedly so in lean subjects.

Palmitate kinetics. In both lean and obese subjects, fasting increased palmitate concentrations (Table 3). Obese subjects

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Lean, n = 9</th>
<th>Obese, n = 9</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24 (21–33)</td>
<td>24 (21-35)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.2 ± 1.8</td>
<td>122.9 ± 4.2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.4 ± 0.4</td>
<td>35.7 ± 0.9</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>57.3 ± 1.2</td>
<td>78.5 ± 2.2</td>
</tr>
<tr>
<td>Weight loss during 72 h of fasting, kg</td>
<td>3.7 ± 0.6</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td>Surface area, m²</td>
<td>1.87 ± 0.03</td>
<td>2.45 ± 0.05</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>10.2 (7.4–14.0)</td>
<td>41.1 (34.1–54.7)</td>
</tr>
<tr>
<td>Upper body fat, %</td>
<td>15 ± 4</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>Lower body fat, %</td>
<td>15 ± 4</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>Muscle tissue in forearm, g</td>
<td>516 ± 17</td>
<td>632 ± 24</td>
</tr>
<tr>
<td>Muscle tissue in forearm, %</td>
<td>84 (75–88)</td>
<td>68 (56–79)</td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>30 (25–35)</td>
<td>33 (30–36)</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone, 10¹ IU/liter</td>
<td>2.4 (1.6–3.7)</td>
<td>2.0 (1.3–3.8)</td>
</tr>
<tr>
<td>Physical activity level, points</td>
<td>7.9 ± 1.1</td>
<td>8.9 ± 1.4</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent ranges. Normally distributed data are presented as means ± SE and were analyzed using the Student’s \( t \)-test. Skewed data were analyzed using the Mann-Whitney rank sum test and are presented as median (range).

Table 2. Levels of hormones, glucose, and free fatty acids in lean and obese subjects after 12 and 72 h of fasting

| Hormones, glucose, and free fatty acids in lean and obese subjects after 12 and 72 h of fasting |
|-------------|-------------|-------------|-------------|-------------|
| Lean 12 h Fast | Lean 72 h Fast | Obese 12 h Fast | Obese 72 h Fast | Interactions BMI × Fasting | Main Effects |
| Insulin, pM | 37 ± 7 | 15 ± 2† | 71 ± 10* | 41 ± 8† | NS | NS |
| C-peptide, pM | 390 ± 39 | 141 ± 15† | 519 ± 54* | 231 ± 39† | <0.001 | <0.001 |
| Free fatty acid, mM | 0.39 ± 0.06 | 1.47 ± 0.09† | 0.55 ± 0.03* | 1.07 ± 0.05† | <0.001 | 0.001 |
| Glucose, mM | 4.9 ± 0.08 | 3.2 ± 0.08† | 4.9 ± 0.13 | 3.7 ± 0.12† | 0.001 | <0.001 |
| Glucagon, ng/liter | 68 ± 7 | 162 ± 16† | 99 ± 9 | 157 ± 18† | <0.05 | NS |
| Cortisol, mM | 109 ± 12 | 128 ± 18 | 104 ± 12 | 94 ± 10 | NS | NS |

Values are means ± SE. *\( P < 0.05 \) compared with lean subjects. †\( P < 0.05 \) compared with 12-h fast. NS, not significant. Insulin C-peptide, glucose, glucagon, and cortisol were measured at \( t = 0 \) and \( t = 240 \), and data are reported as a mean of the two measurements. Free fatty acid levels were measured in triplicate at \( t = 210-240 \) and presented as a mean of those measurements.

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displayed higher palmitate concentration (~35%) than lean subjects after 12 h of fasting (158 ± 7 vs. 118 ± 19 μM), however, after 72 h of fasting this was reversed. Whole body palmitate fluxes were significantly higher in obese than lean subjects (256 ± 30 vs. 139 ± 23 μmol/min, P < 0.001) and increased during prolonged fasting in both groups (2.2-fold in obese subjects vs. 2.5-fold in lean subjects). However, obese subjects had an increased fat mass (41.1 vs. 10.2 kg, P < 0.001, Table 1), and consequently, an approximately 2.3-fold lower palmitate flux/kg fat mass than lean subjects after both 12 and 72 h of fasting.

Protein and amino acid forearm and whole body kinetics. Phenylalanine forearm balances per 100 ml of forearm tissue were −2.2 ± 0.4 (obese) vs. −5.6 ± 1.2 nmol·100 ml tissue−1·min−1 (lean) after 12 h of fasting and −2.5 ± 0.6 (obese) vs. −5.6 ± 0.4 nmol·100 ml tissue−1·min−1 (lean) after 72 h of fasting, implying that the net phenylalanine forearm release, reflecting net forearm protein loss, was reduced by ~60% in obese subjects (ANOVA main effect of BMI P = 0.002) (Table 3). This finding was caused by the fact that arterio-venous concentration gradients of phenylalanine were comparable (around 2–3 μM) despite the presence of forearm blood flow rates that were 2.5- to 3-fold higher in lean than obese subjects. Phenylalanine forearm balances were not affected by fasting. Both forearm phenylalanine rates of appearance [Ra ϕphe, reflecting protein breakdown, 8.2 ± 0.8 (obese) vs. 17.6 ± 0.6 nmol·100 ml tissue−1·min−1 (lean), P < 0.001] and disappearance [Rd ϕphe, reflecting protein synthesis, 5.9 ± 0.7 (obese) vs. 12.0 ± 1.4 nmol·100 ml tissue−1·min−1 (lean), P = 0.004] were significantly lower in obese than lean subjects. Fasting for 72 h decreased Ra ϕphe and Rd ϕphe in both groups; post hoc tests showed that this effect was significant only in lean subjects. Forearm plasma flow rates were 2.5- to 3-fold higher in lean than obese subjects, and increased slightly with fasting.

Whole body phenylalanine and tyrosine fluxes [per kilogram of total body weight (TBW)] were significantly lower in obese subjects (30.1 ± 1 vs. 39.4 ± 1.5 μmol·kg TBW−1·h−1, P < 0.001) with no differences between 12 and 72 h of fasting (Table 3). Phenylalanine hydroxylation to tyrosine was significantly reduced by 72 h of fasting but was not significantly different between the two groups. Likewise, urea fluxes per kilogram of TBW (248 ± 16 vs. 297 ± 11 μmol·kg TBW−1·h−1) and urinary nitrogen excretion rates per kilogram of TBW were significantly decreased in obese subjects (ANOVA main effect of BMI P = 0.001) and were not affected by 72 h of fasting.

Regulation of lipolysis in adipose tissue. Protein expression of HSL was not affected by BMI or fasting (Figs. 1 and 2). Phosphorylation levels of HSL at Ser552, Ser554, and Ser560 were significantly lower in obese subjects overall. Although we found no significant interactions between the effect of BMI and fasting, HSL p-Ser552 and p-Ser560 in lean subjects were increased by 2- to 2.5-fold after 72 h of fasting, whereas the

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**Table 3. Whole body and forearm substrate concentrations and fluxes**

<table>
<thead>
<tr>
<th></th>
<th>Lean 12 h Fast</th>
<th>Obese 12 h Fast</th>
<th>Interactions BMI × Fasting</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitate concentration, μmol/liter</td>
<td>118 ± 19</td>
<td>381 ± 26†</td>
<td>158 ± 7*</td>
<td>276 ± 15†</td>
</tr>
<tr>
<td>Palmitate flux, μmol/min</td>
<td>139 ± 23</td>
<td>342 ± 38†</td>
<td>256 ± 30*</td>
<td>572 ± 106†</td>
</tr>
<tr>
<td>Palmitate flux per fat mass, μmol·min−1·kg fat−1</td>
<td>14.5 ± 4.8</td>
<td>35.5 ± 11.8†</td>
<td>6.3 ± 2.1*</td>
<td>14.2 ± 4.7†</td>
</tr>
<tr>
<td>Palmitate flux per lean mass, μmol·min−1·kg LBM−1</td>
<td>2.4 ± 0.4</td>
<td>6.0 ± 0.6†</td>
<td>3.3 ± 0.5</td>
<td>7.4 ± 1.5†</td>
</tr>
<tr>
<td>Phenylalanine flux, μmol·kg TBW−1·h−1</td>
<td>39.4 ± 1.5</td>
<td>39.1 ± 1.5</td>
<td>30.1 ± 1.0*</td>
<td>30.5 ± 0.7*</td>
</tr>
<tr>
<td>Phenylalanine flux per LBM, μmol·kg LBM−1·h−1</td>
<td>47.3 ± 2.2</td>
<td>44.0 ± 1.2</td>
<td>47.5 ± 1.8</td>
<td>46.3 ± 1.2</td>
</tr>
<tr>
<td>Tyrosine flux, μmol·kg TBW−1·h−1</td>
<td>28.1 ± 0.6</td>
<td>27.4 ± 1.5</td>
<td>25.6 ± 1.3</td>
<td>22.7 ± 0.9*</td>
</tr>
<tr>
<td>Rate of phenylalanine conversion to tyrosine, μmol·kg TBW−1·h−1</td>
<td>3.4 ± 0.6</td>
<td>2.4 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Whole body urea flux, μmol·kg TBW−1·h−1</td>
<td>297 ± 11</td>
<td>349 ± 18</td>
<td>248 ± 16</td>
<td>247 ± 17*</td>
</tr>
<tr>
<td>U-nitrogen, g·kg TBW−1·24 h−1</td>
<td>0.21 ± 0.02</td>
<td>0.19 ± 0.03</td>
<td>0.15 ± 0.01*</td>
<td>0.14 ± 0.02*</td>
</tr>
</tbody>
</table>

Forearm metabolism | 2.6 ± 0.1 | 3.1 ± 0.4 | 0.9 ± 0.1* | 1.2 ± 0.2* | <0.001 | <0.05 |
| Phenylalanine balance, nmol·100 ml forearm tissue−1·min−1 | −5.6 ± 1.2 | −5.6 ± 0.4 | −2.2 ± 0.4 | −2.5 ± 0.6* | NS | <0.01 | NS |
| Phenylalanine balance, nmol·100 ml muscle−1·min−1 | −6.3 ± 1.8 | −6.6 ± 0.7 | −3.1 ± 0.6 | −3.6 ± 1.2* | NS | <0.05 | NS |
| Phenylalanine Ra, nmol·100 ml forearm tissue−1·min−1 | 17.6 ± 0.6 | 12.2 ± 1.3| 8.2 ± 0.8* | 6.3 ± 0.7* | NS | <0.001 | <0.01 |
| Phenylalanine Rd, nmol·100 ml forearm tissue−1·min−1 | 12.0 ± 1.4 | 6.5 ± 1.1† | 5.9 ± 0.7* | 3.8 ± 0.6 | NS | <0.01 | <0.01 |
| Phenylalanine arterial concentration, μmol/liter | 33 ± 2 | 31 ± 2† | 40 ± 3* | 35 ± 2† | NS | <0.05 | <0.001 |
| Phenylalanine venous concentration, μmol/liter | 36 ± 2 | 34 ± 2† | 42 ± 3* | 38 ± 2† | NS | NS | <0.001 |

Values are means ± SE. *P < 0.05 compared with lean subjects. †P < 0.05 compared with 12-h fast. Tracer enrichments and concentrations were measured in triplicate at t = 210–240 and presented as a mean of those measurements. TBW, total body weight; LBM, lean body mass; Ra, rate of appearance; Rd, rate of disappearance.
increase in obese subjects was much smaller (30–45%). Post hoc tests revealed that the fasting-induced phosphorylation increase was significant in lean subjects at HSL p-Ser552 \( (P < 0.01) \) and showed a trend toward significance in lean subjects on HSL p-Ser554 \( (P = 0.077) \) and HSL p-Ser650 \( (P = 0.071) \), whereas the changes in obese subjects were not significant.

ATGL mRNA levels were lower in obese than lean subjects, the difference being most pronounced after 12 h of fasting, and levels were significantly reduced by 72 h of fasting in both lean and obese subjects. Protein expression of ATGL was lower in obese than in lean subjects without any effect of fasting.

The mRNA levels of the ATGL inhibitor G0S2 displayed significant interaction between BMI and fasting, indicating that the effect of prolonged fasting on G0S2 was different between the two groups. Lean subjects exhibited a \( \sim 75\% \) reduction compared with \( \sim 35\% \) in obese subjects, with a lower G0S2 mRNA level before and a higher level after prolonged fasting in obese subjects. The protein content of G0S2 was significantly lower in obese subjects and decreased during prolonged fasting in both groups. Both mRNA and protein levels of CGI58, an ATG5 activator, were significantly lower in obese compared with lean subjects. CGI58 mRNA showed a significant interaction, reflecting a greater increase during 72 h of fasting in lean (\( \sim 100\% \)) than obese subjects (\( \sim 75\% \)). CIDEC mRNA and protein levels were significantly lower in obese subjects and increased with 72 h of fasting, albeit only significantly in lean subjects. CIDEA showed a significant interaction between BMI and fasting effects on both mRNA and protein expression. A post hoc test of CIDEC mRNA revealed lower levels in obese subjects after 12 of fasting and a reduction in CIDEC mRNA with 72 h of fasting in both groups, the reduction being greater in lean than obese subjects (\( \sim 60\% \) vs. \( \sim 35\% \)). A post hoc test showed a significant increase with 72 h of fasting in obese subjects only.

**Regulation of protein metabolism in muscle.** Protein expression of mTOR was \( \sim 20\% \) higher in lean subjects than in obese subjects (Figs. 3 and 4). Phosphorylation of Ser2448 on mTOR was similarly higher in lean subjects, and was significantly reduced by 72 h of fasting. The ratio of mTOR p-Ser2448 vs. total mTOR was not affected by BMI, but the levels were reduced \( \sim 20\% \) by 72 h of fasting.

Furthermore, expression of eukaryotic translation initiation factor 4E binding protein 1 (4EBP1), a downstream protein synthetic target of mTOR, was significantly higher in lean than obese subjects. In lean subjects, phosphorylation of 4EBP1 was significantly decreased after 72 h of fasting compared with the 12-h fast (observed by \( \sim 85\% \) increased nonphosphorylated 4EBP1), indicating loss of downstream mTOR signaling in lean subjects, whereas obese subjects exhibited a smaller, nonsignificant reduction, resulting in significantly lower phosphorylated 4EBP1 levels in lean subjects after prolonged fasting. The ratio of phosphorylated vs. total 4EBP1 was not significantly different between the groups, but decreased more in lean than obese subjects during 72 h of fasting.

The mRNA and protein levels of ULK1, which initiates autophagy, increased significantly with 72 h of fasting, and were not different between groups. Phosphorylation of ULK1 Ser757 (which inhibits ULK1 action) was not significantly affected by BMI or fasting, but the ratio of ULK1 p-Ser757 to total ULK1 decreased with fasting.

LC3 and p62 are markers of autophagy. The mRNA levels of p62 increased significantly with fasting in both groups. Protein expression of p62 displayed significant interaction between BMI and fasting, reflecting that lean subjects exhibited a greater increase in p62 with 72 h of fasting (\( \sim 50\% \)) than obese subjects (\( \sim 15\% \)). We did not find any effect of body composition or fasting on LC3 mRNA or protein levels of LC3B-II compared with LC3B-I.

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**Fig. 1.** Hormone-sensitive lipase (HSL) protein expression and phosphorylation assessed by Western blotting in subcutaneous adipose tissue (AT) from lean and obese subjects after 12 and 72 h of fasting. Data were ln-transformed to achieve normal distribution. Graphs shown means \( \pm \) SE of ln-transformed data. A: protein expression of HSL was not affected by BMI or fasting. B: HSL p-Ser552 was significantly lower in obese subjects [body mass index (BMI) \( P < 0.001 \)] and increased after 72 h of fasting (fasting \( P = 0.011 \)). Lean subjects exhibited a greater increase (\( P < 0.01 \)) after 72 h of fasting compared with obese subjects (not significant). C: HSL p-Ser650 was significantly lower in obese subjects (BMI \( P < 0.001 \)). The increase in lean subjects with 72 h of fasting was greater than in obese subjects. D: HSL p-Ser554 was significantly lower in obese subjects (BMI \( P < 0.001 \)) and increased significantly after 72 h of fasting (fasting \( P < 0.01 \)), slightly more in lean subjects than obese subjects. Representative Western blots have been cut to remove lanes showing samples obtained during hyperinsulinemic euglycemic clamp conditions. All representative blots show blots from one test subject and arise from the same membrane. ** \( P < 0.01 \), *** \( P < 0.001 \).
Fig. 2. Messenger RNA and protein expressions of adipose triglyceride lipase (ATGL) and regulators of ATGL activity assessed by PCR and Western blotting in subcutaneous AT from lean and obese subjects after 12 and 72 h of fasting. Protein data were ln-transformed to achieve normal distribution and protein; graphs show means ± SE of ln-transformed data. mRNA data were normally distributed, and graphs show original data as means ± SE. A: ATGL mRNA expression was lower in obese than lean subjects (BMI effect $P = 0.050$), especially after the 12-h fast. ATGL mRNA was decreased in both groups with 72 h of fasting (fasting effect $P < 0.001$). B: total ATGL protein was significantly lower in obese than lean subjects (BMI effect $P < 0.001$). C: at the G0S2 mRNA level we found a significant interaction between BMI and fasting effects (BMI × fasting $P < 0.001$). D: G0S2 protein was lower in obese subjects (BMI effect $P < 0.05$) and decreased after 72 h of fasting (fasting effect $P < 0.05$). E and F: both mRNA and protein levels of CGI-58 were significantly lower in obese than lean subjects (all $P$ values $< 0.01$). CGI-58 mRNA showed a significant interaction (BMI × fasting $P < 0.001$) but it increased significantly in both groups with fasting ($P$ values $< 0.003$). G and H: CIDEA mRNA and protein levels were significantly lower in obese than lean subjects (BMI effects $P < 0.001$) and increased significantly with 72 h of fasting (fasting effect on mRNA $P < 0.05$; protein $P < 0.001$). I and J: there was a significant interaction of both CIDEC mRNA (BMI × fasting $P < 0.05$) and protein expression (BMI × fasting $P < 0.05$). Representative Western blots have been cut to remove lanes showing samples obtained during hyperinsulinemic euglycemic clamp conditions. All representative blots show blots from one test subject and arise from the same membrane. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

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In addition, we assessed the proteasome pathway of protein degradation by monitoring the expression of the two ubiquitin E3 ligases, muscle-specific muscle ring finger 1 (MuRF1) and atrogin (also named muscle atrophy box protein/MAFbx). We found that atrogin mRNA increased with fasting in both groups. MuRF1 displayed a significant interaction, reflecting that mRNA levels increased significantly with 72 h of fasting in lean subjects only.

DISCUSSION

This study demonstrates that whole body lipolytic fluxes are increased in obese subjects and increased by prolonged fasting. In obese subjects increased lipolysis is driven by a large fat mass with low expression of regulators of lipolysis in AT, whereas the driving force during fasting appears to be distinctly increased phosphorylation of HSL and decreased protein levels of the ATGL inhibitor G0S2. In addition, we found that compared with lean subjects, obese subjects are characterized by a markedly reduced forearm phenylalanine release, and both decreased protein breakdown and synthesis in skeletal muscle of the forearm together with reduced urea fluxes and urine nitrogen excretion rates after both 12 and 72 h of fasting. Muscle protein expressions of mTOR and 4EBP1 were decreased in obese subjects, and mTOR phosphorylation and signaling decreased with fasting in both groups, whereas ULK1 protein and mRNA were increased.

Obese subjects are characterized by increased lipolysis per kilogram of LBM but decreased lipolysis per kilogram of fat mass (7) in addition to decreased maximal lipolytic capacity (31). We found that whole body palmitate fluxes were close to twofold higher in obese subjects despite their having higher insulin levels. Per fat mass unit palmitate fluxes were, however, lower in obese subjects after both 12 and 72 h of fasting, indicating that obesity is a condition with a large fat mass overriding low lipolytic activity. Obese subjects in general have greater fat cell size, but large and small fat cells have similar basal rates of lipolysis when expressed per unit of fat mass (14). It has been reported that AT vascularization and oxygenation are decreased in obesity and that this defect correlates with AT metabolic flexibility (28).

Phosphorylation of Ser552 is believed to promote the translocation of HSL from the cytosol to the lipid droplets, where triglycerides are stored, whereas phosphorylation of Ser650 is critical for activation of intrinsic enzymatic activity. Conversely, phosphorylation on Ser554 by AMP kinase inhibits HSL activation (25). Our data showed that lean subjects display higher HSL phosphorylation levels on all three phos-
Fig. 4. mRNA expression of ULK1, p62, LC3, atrogin, and MuRF1; protein expression of ULK1, p62, and LC3; and phosphorylation of ULK1 at Ser757 assessed by PCR and Western blotting in skeletal muscle from lean and obese subjects after 12 and 72 h of fasting. Data are presented as means ± SE. A and B: mRNA and protein levels of ULK1 increased significantly with 72 h of fasting (fasting effect $P < 0.001$), and were not different between groups using body mass index (BMI). C and D: phosphorylation of ULK1 Ser757 was not significantly affected by BMI or fasting, but the ratio of ULK1 p-Ser757 to total ULK1 was significantly decreased with fasting (fasting effect $P < 0.001$). E: p62 mRNA levels increased significantly with fasting (fasting effect $P < 0.001$). F: there was significant interaction between effects of BMI and fasting (BMI × fasting $P < 0.05$) on p62 protein expression, reflecting a greater increase in p62 with 72 h of fasting in lean than obese subjects. G and H: there was no effect of BMI or fasting on LC3 mRNA or protein levels of LC3B-II compared with LC3B-I. I: atrogin mRNA increased significantly with fasting (fasting effect $P < 0.001$) with no BMI effect. J: there was significant interaction of MuRF1 (BMI × fasting $P < 0.05$), revealing that mRNA levels increased significantly with 72 h of fasting in lean subjects ($P < 0.001$) but not in obese subjects. Representative Western blots have been cut to remove lanes showing samples obtained during hyperinsulinemic euglycemic clamp conditions. All representative blots show blots from one test subject and arise from the same membrane. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

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phorylation sites than obese subjects and show a greater increase in phosphorylation on Ser^{552} and Ser^{650} after 72 h of fasting than obese subjects, which is compatible with increased lipolysis per fat mass unit. Total HSL levels were, in our study, not significantly affected by BMI or fasting, whereas other studies have shown reduced levels of HSL protein in obesity (12, 30, 34).

ATGL has emerged as the principal activator of the initial hydrolytic step of lipolysis (43). The regulation of ATGL activity is complex and not fully understood, and results from studies regarding ATGL mRNA levels and protein expression in obesity are conflicting (25). Our data show that mRNA and protein levels of ATGL were significantly lower in obese than in lean subjects. In addition, mRNA and protein levels of CGI58, an ATGL coactivator (13), were significantly lower in obese subjects. These events, together with the blunted stimulation of Ser^{552} and Ser^{650} HSL phosphorylation, may explain why lipolysis per gram of fat is decreased in obese subjects. On the other hand, the mRNA and protein levels of the specific ATGL inhibitor G0S2 (42, 44), as well as protein and mRNA of CIDEA and CIDEC, which are putative inhibitors of lipolysis (25), were all decreased in obese subjects, providing a potential compensation for the reduced HSL and ATGL-mediated lipolytic drive. These distinct differences between obese and lean subjects persisted during prolonged fasting. Data on HSL and ATGL content and expression in obesity are generally conflicting, which may to a large extent reflect regional metabolic differences between lower body, upper body, and visceral fat, and many studies have not addressed the role of important coregulators; here we report detailed differences in the regulation of lipolysis in abdominal AT between lean and obese subjects.

We observed more than a doubling of palmitate fluxes in both lean and obese subjects who fasted. Ser^{552} HSL phosphorylation increased and the content of G0S2 protein and mRNA decreased, which is indicative of increased lipolysis. The complexity of the regulation of lipolysis is, however, evidenced by the fact that ATGL and CGI58 mRNA and protein content decreased and CIDEA mRNA and protein increased, thus counteracting stimulation of lipolysis. A previous study has reported decreased G0S2 protein and mRNA levels together with increased protein and unaltered mRNA ATGL levels during fasting (26).

We found that both protein breakdown and synthesis per 100 ml of forearm tissue were reduced in obese subjects compared with lean subjects, and although lean subjects demonstrated decreased protein synthesis and breakdown after 72 h of fasting, obese subjects did not exhibit similar reductions. These findings are supported by a previous report showing that leucine fractional synthesis rates in muscle biopsies is not reduced during weight loss induced by dietary calorie restriction in obese, older adults (39).

A recent study compared leg muscle protein metabolism in older obese and lean subjects under highly complex metabolic conditions of insulin, glucagon, and somatostatin administration and found similar indices of protein synthesis and breakdown and intramyocellular protein metabolic signaling in the two groups (19). The discrepancy with our results could relate to the metabolic complexity of the setup altering the concentrations of protein metabolic hormones such as insulin, glucagon, and growth hormone.

As reported earlier, forearm blood flows were lower in obese than in lean subjects (29), a finding that could relate to a relatively higher amount of fat in the forearms of obese than lean subjects and the reduced net protein loss across the forearm in our obese subjects is caused by a combination of low blood flows and unaltered arterio-venous phenylalanine concentration gradients. The observed decrease in blood flows contributes substantially to the reduced protein synthesis, breakdown, and net protein loss across the forearm in our study. Not all studies have shown decreased blood flow in obese subjects, but many of these studies have reported whole limb blood flows, which does not take the increased obesity-associated limb volume, caused by increased muscle and fat mass, into account. In our study, calculated forearm masses were 930 g in obese and 615 g in lean subjects, with increases in both muscle and fat masses. In an attempt to correct for differences in forearm composition, we calculated the individual forearm lean tissues in percent of total forearm mass (muscle percentage) and recalculated our phenylalanine kinetic data per muscle tissue percentage. Although the differences between lean and obese subjects were reduced by this correction, the statistical differences persisted. It is plausible that the higher plasma insulin concentrations in obese subjects may have contributed to restriction of protein synthesis, although we failed to observe any difference between groups in intramyocellular indices of protein breakdown and autophagy.

At the whole body level, tyrosine, phenylalanine and urea fluxes, and urine-nitrogen excretion rates calculated per TBW were lower in obese subjects, indicating that obese subjects have reduced whole body release of protein compared with lean subjects, which may promote muscle protein conservation. When fluxes per LBM were calculated these differences disappeared, but as mentioned above, this correction ignores the fact that AT may account for close to 10% of whole body protein breakdown (29).

The activity of mTOR, which promotes protein synthesis, is regulated by phosphorylation, and phosphorylation of mTOR at Ser^{2448} is essential for mTOR downstream regulation of ribosomal mRNA translation. In accordance with previous findings, we found reduced skeletal muscle protein synthesis (R_{p,b}), in parallel with decreased mTOR Ser^{2448} phosphorylation after 72 h of fasting (38). Additionally, we found that protein synthesis (R_{p,b}), total mTOR expression and phosphorylation of mTOR p-Ser^{2448} to mTOR were reduced in obese subjects.

We therefore examined 4EBP1, which is a downstream target of mTOR, and found that protein levels of 4EBP1 were significantly lower in obese than lean subjects. Also, phosphorylation levels of 4EBP1 were significantly decreased with 72 h of fasting in lean but not in obese subjects, in line with the observed significantly more pronounced reduction in muscle protein synthesis in lean subjects after 72 h of fasting.

Muscle protein breakdown (R_{b,p,b}) decreased by ~30% in lean subjects compared with ~23% in obese subjects with 72 h of fasting, and this decrease was significant only in lean subjects. Obese subjects still maintained a lower protein breakdown than lean subjects after both 12 and 72 h of fasting.

The regulation of protein breakdown and autophagy involves a series of other complex reactions and, as reviewed elsewhere (33), there are several, at times seemingly counteractive and counterringuive, interdependencies between mTOR,
AMP, andULK kinases. In our hands, fasting increased ULK1 mRNA and protein levels and decreased ULK1 Ser757 phosphorylation to the same extent in both groups, which is not compatible with the observed decrease in protein breakdown. Adding to this discrepancy, we observed higher mRNA levels of the two E3 ubiquitin ligases, atrogin and MuRF1. On the contrary, p62 protein, which is consumed during autophagy (4, 5), increased after fasting, which is suggestive of decreased protein breakdown, as observed, although increased synthesis of p62 may contribute to this, as is suggested by the increased mRNA levels.

Our study clearly has limitations. Whenever comparisons are made between groups with different body composition the investigator faces the dilemma of how to express results. Because AT is an active metabolic component of the body we chose to express results per kilogram of TBW. This implies that metabolic fluxes may be underestimated in obese subjects to the extent that AT is less metabolically active than LBM, which poses specific problems regarding protein metabolism. It is, however, important to underline that the differences we observed in muscle protein metabolism between obese and lean subjects persisted after correction for the excess forearm fat component measured with DEXA scans. Furthermore, urea fluxes and urinary nitrogen excretion rates were decreased in obese subjects, although statistical significance was lost when calculated per lean body weight. Finally, as mentioned above, the regulation of intracellular signaling in muscle and AT is a highly complex field, which is subject to both time and tissue dependencies, implying that our results could have been different if we had performed sampling at other time points or from other muscles or ATs. It has, for instance, been shown that muscle protein synthesis may decrease despite elevated intramyocellular synthetic signals after an oral protein load, implying that additional downstream or alternative signals may modulate metabolic reactions in a discordant manner (3).

Likewise, the quantity or phosphorylation of an enzyme depends on coactivation and/or coinhibition and does not necessarily reflect its activity.

Our study was designed to test whether obese subjects display increased lipolysis and decreased muscle amino acid release, and whether these adaptations persist during prolonged fasting. We found a close to twofold increase in whole body lipolysis measured with labeled palmitate in the presence of a fourfold increase in fat mass, implying that lipolysis per fat mass unit is decreased in obese subjects. Fasting resulted in more than doubled palmitate fluxes in both groups. We also found decreased net protein release and decreased protein breakdown and synthesis in skeletal muscles of the forelimb and decreased ureagenesis in obese subjects; these differences persist after 72 h of fasting.

We also tested whether such adaptations could be mediated by modifications in adipose tissue ATGL- and HSL-related signaling and alterations in muscle mTOR and ULK1-mediated signaling to protein synthesis and autophagy. Consistent with the decreased lipolysis per fat mass unit, we found decreased HSL phosphorylation and decreased ATGL protein and mRNA contents in obese subjects. Fasting in general resulted in higher HSL phosphorylation and lower G0S2 mRNA and protein. The lower muscle protein turnover in obese subjects could relate to inhibition of mTOR signaling.

Overall, our data suggest that an increased fat mass and an increased whole body lipolytic rate may protect and restrict loss of vital protein during calorie deprivation in obese subjects.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES
