# Obesity appears to be associated with altered muscle protein synthetic and breakdown responses to increased nutrient delivery in older men, but not reduced muscle mass or contractile function.

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**Abstract**

Obesity is increasing, yet despite the necessity to maintain muscle mass and function with age, the effect of obesity on muscle protein turnover in older adults remains unknown. Eleven obese (BMI 31.9 ±1.1) and 15 healthy weight (HW; BMI 23.4 ±0.3) older men (55-75 years old) participated in a study that determined muscle protein synthesis (MPS) and leg protein breakdown (LPB) under post-absorptive (hypoinsulinaemic euglycaemic clamp) and post-prandial (hyperinsulinemic hyperaminoacidaemic euglycaemic clamp) conditions. Obesity was associated with systemic inflammation, greater leg fat mass, and patterns of mRNA expression consistent with muscle deconditioning, whilst leg lean mass, strength and work done during maximal exercise were no different. Under post-absorptive conditions, MPS and LPB were equivalent between groups, while insulin and amino acid administration increased MPS in only HW subjects and was associated with lower leg glucose disposal (LGD, 63%) in obese. Blunting of MPS in the obese was offset by an apparent decline in LPB, which was absent in HW subjects. Lower post-prandial LGD in obese subjects and blunting of MPS responses to amino acids suggests obesity in older adults is associated with diminished muscle metabolic quality. However this doesn't appear to be associated with lower leg lean mass or strength.

**Introduction**

Aging is strongly associated with a decline in muscle mass and strength (1). This has been in part attributed to the failure of protein nutrition to increase the rate of muscle protein synthesis (MPS) to that observed in the young (2), and the inability of hyperinsulinaemia to inhibit leg protein breakdown (LPB) in older people (3). However, even after correcting for muscle mass loss, ageing has been allied with a decline in muscle strength and metabolic quality (4), signifying that physiological drivers other than muscle mass must contribute to weakness and diminished quality of muscle in older adults (5).

It is widely reported that the prevalence of obesity is increasing in the developed world across all ages, including in older adults (6). Of concern, a longitudinal study in older adults showed that the temporal decline in lean mass and leg extension strength relative to leg mass (the latter used as an index of muscle quality) could be predicted by the degree of fat mass evident at time of recruitment (7). Furthermore, this relationship remained even when differences in physical activity levels were taken into account (8). The implication that older adults may be particularly at risk of accelerated muscle deterioration from obesity is supported by a cross-sectional study involving 2,039 older men and women showing that the combination of low muscle strength and obesity was associated with poorer physical function than either in isolation (9). Crucially, whether the derangements in MPS and LPB described above for normal weight older adults (2; 3) are further exacerbated in obese older adults is unknown and forms the focus of the current study.

Several reports provide credence that MPS and LPB may be negatively affected in obese individuals. When visceral fat mass is enlarged, plasma concentrations of TNFα, interleukin-6 (IL-6), resistin and leptin appear increased (for a review see (10)). Furthermore, increased

plasma IL-6, c-reactive protein (CRP) and TNFα concentrations in older adults have been associated with the development of insulin resistance (11) and the decline of muscle mass and strength (12; 13). Moreover, in pre-clinical models characterised by systemic increases in TNFα and IL-6, cytokine mRNA expression in muscle is markedly elevated and processes involved in the promotion of protein synthesis inhibited in parallel to enhanced proteolysis (14; 15).

Therefore, the aim of this study was to investigate if the response of MPS and LPB to experimentally induced post-absorptive and post-prandial conditions was different in obese older men compared to healthy weight older men, and if so, whether these responses could underlie any deficits in muscle mass and contractile function observed in obese older individuals.

## Research Design and Methods

*Study participants*

Men between the ages of 55 and 75 were recruited and assigned to either a healthy weight (control) group if they were found to have a BMI < 26 and a serum CRP < 1.35 $\mu g.ml^{-1}$ (n=15) or obese group if a BMI > 29 and CRP > 1.35 $\mu g.ml^{-1}$ (n=11). This benchmark was based on previous reports that serum CRP concentrations greater than 1.35 $\mu g.ml^{-1}$ associate with increased levels of IL-6 and TNFα in obese individuals (16). All other individuals not meeting these criteria were excluded from participating. Subjects underwent a medical examination and were unable to participate if they had: type-II diabetes, previous diagnosis of cancer, unresolved hypertension, cardiovascular disease, on-going musculoskeletal complaints, frailty (underweight and self-reporting of exhaustion, weakness, slowness, and low physical activity levels; (17)) or any other chronic condition. Furthermore, subjects were not allowed to participate if they smoked, were trying to lose weight, were participating in regular organised sports, or taking anti-inflammatories, anti-depressants or statin medication. Characteristics of included subjects are presented in Table 1. Informed written-consent was obtained from volunteers and the study was approved by the University of Nottingham Medical School Ethics Committee and conducted in accordance with the Declaration of Helsinki.

*Muscle function protocol*

Subjects were instructed to refrain from alcohol and exercise for 48 h prior to each visit and attend the laboratory at 08:00 in a fasted state. On arrival, a whole-body dual-energy x-ray absorptiometry (DXA) scan was performed to assess lean and fat masses (Lunar Prodigy; GE Healthcare). Afterwards, a subset of volunteers (10 healthy weight and 8 obese subjects)

underwent isometric and isokinetic leg muscle function measurements as described in detail elsewhere (18).

*Metabolic study protocol*

A minimum of 72 h was allowed to elapse from the muscle function assessment visit before subjects returned to the laboratory to undergo detailed assessment of multiple whole-body and muscle metabolic parameters utilising an insulin-clamp protocol described previously (18). In short, a 240 min euglycaemic (4.5 $mmol.l^{-1}$) insulin clamp began at t=0 with an insulin infusion rate of 0.6 $mU.m^{-2}.min^{-1}$ to maintain serum insulin concentrations similar to the fasting condition (~ $5mU.l^{-1}$) with octreotide (30 $ng.kg^{-1}.min^{-1}$) and glucagon (15 $ng.kg^{-1}.h^{-1}$) administered to block endogenous insulin production and maintain serum glucagon concentrations, respectively (Figure 1). At t=120 the insulin infusion rate was increased to 15 $mU.m^{-2}.min^{-1}$ to achieve a serum insulin concentration equivalent to the post-prandial state (~40 $mU.l^{-1}$). In parallel a mixed amino acid infusion (10 $g.h^{-1}$; Glamin®, Fresenius Kabi, Germany) commenced which was maintained for the remaining 120 min of the study period. Throughout the entire 240 min period of the insulin clamp, a primed constant infusion of the stable-isotope labelled amino acids [1,2-$^{13}C_2$]-leucine and [ring-$D_5$]-phenylalanine were administered (Figure 1).

Muscle biopsies were obtained from the vastus lateralis by percutaneous needle biopsy at t=0, 120 and 240 min. Arterialised-venous (a-v) blood samples were obtained at 5 min intervals for the determination of blood glucose (YSI2300 automated analyser, Yellow Springs, USA) and serum insulin concentrations by enzyme-linked immunosorbent assay (ELISA; DRG Diagnostics, Germany)); an additional sample taken at t=0 was used to determine circulating TNFα, IL-6, resistin, adiponectin and leptin concentrations by ELISA (R&D Systems Inc.,

UK). Femoral venous blood samples taken in tandem with a-v blood samples at regular intervals were analysed for blood glucose concentrations to determine leg glucose disposal (LGD) between the period 210 to 240 min, reflecting steady-state under fed conditions. Arterialised-venous and femoral venous blood samples were obtained at t=0, 75, 90, 105, 120, 195, 210, 225 and 240 min for the determination of [1,2-$^{13}C_2$]-leucine and [ring-$D_5$]-phenylalanine enrichment as previously described (19). Femoral artery blood flow was determined in the contralateral limb at these same time points using Doppler ultrasound (Aplio XV, Toshiba). Prior to the end of the 0.6 mU.m$^{-2}$.min$^{-1}$, and 15 mU.m$^{-2}$.min$^{-1}$ clamps, the respiratory exchange ratio (RER) was determined using a ventilated-hood indirect calorimetry system (GEM, GEM Nutrition Ltd, UK).

*Muscle protein synthesis*

Myofibrillar proteins were extracted from ~30 mg of muscle tissue as previously described (19). Following extraction, proteins were hydrolysed into their constituent amino acids via acid hydrolysis and purified using ion exchange chromatography (20). The resultant amino acids were derivatised as their N-acetyl-N-propyl esters and the incorporation of [1,2-$^{13}C_2$]-leucine into protein determined by capillary gas chromatography combustion isotope ratio mass spectrometry (Delta-plus XL; Thermo Fisher Scientific, UK) according to established methods (20). The rate of MPS was determined by measuring the incorporation of [1,2-$^{13}C_2$]-leucine in successive biopsies, with the labelling of blood α-KIC (alpha-ketoisocaproate) used as a surrogate measure for the immediate precursor for protein synthesis, leucyl-t-RNA (20).

*Leg protein turnover*

Whole-blood was used to determine labelling (atom % excess) and concentration of a-v and venous phenylalanine. Blood samples were precipitated with perchloric acid and the

supernatant neutralised prior to purification by cation exchange chromatography. The subsequent eluent was dried under nitrogen prior to derivatisation of amino acids to their tert-butyldimethylsilyl derivatives using standard techniques (21). Concentrations and enrichments were determined by gas-chromatography mass-spectrometry (Trace DSQ, Thermo Scientific, UK) with the use of norleucine and d2-phenylalanine as internal standards. For each 120 min post-absorptive and post-prandial period, the values for enrichment and concentration were determined from the mean of 4 separately analysed samples, collected over the final 45 min. Leg protein synthesis (LPS) was calculated as the disappearance of phenylalanine into the leg and LPB as the arteriovenous dilution of [ring-$D_5$]-phenylalanine as previously described (20).

*Gene expression analysis*

Total RNA was extracted from muscle biopsy specimens and cDNA synthesised using standard protocols (18). mRNA expression levels for 90 genes spanning carbohydrate metabolism, fat metabolism, insulin signalling and proteolysis were examined via the use of low-density microfluidic cards (Applied Biosystems, USA; see Table 2 for a comprehensive list of genes examined) and analysed on an Applied Biosystems 7900 Real-Time PCR system. Data was normalised to the geometric average of the housekeeping genes: alpha-actin, β2-microglobulin, 18S and RNA polymerase 2A, with suitability of the housekeeping genes confirmed by the RealTime StatMiner software (Integromics, Spain). Gene expression was calculated via use of the $2^{-\Delta\Delta Ct}$ method and significance determined by the linear modelling approach (LIMMA). The false discovery rate was assessed via the Benjamin and Hochberg method (22).

*Immunoblotting*

Cytosolic proteins were extracted from 30 mg of muscle tissue and analysed by western blot using established methods (23). Membranes were incubated overnight at 4°C with a polyclonal antibody for either total or phosphorylation-specific forms of AKT1 ($Thr^{308}$) or mTOR ($Ser^{2448}$; Cell Signaling Technology Inc., USA) or as a loading control, beta-actin (Sigma, UK). Following washing in TBS-T, membranes were probed with a fluorescently-labelled anti-mouse or anti-rabbit secondary antibody as appropriate (Dylight® conjugate, Cell Signaling Technology Inc., USA) and imaged on an electronic image acquisition system (Odyssey CLx, Licor Biosciences, USA). Band densities were determined using proprietary software supplied with the imaging system and normalised to the loading control.

*Statistics*

Statistical differences in subject characteristics, body composition, muscle functional data, plasma adipokine concentrations, and area under the curve data for serum insulin and glucose disposal rate (GDR) were determined using unpaired 2-tailed Student's t-tests. Differences in RER, femoral blood-flow, protein turnover measurements and western blot data were determined using a two-way ANOVA with repeated measures. When a significant interaction between main effects was observed, a Student's t-test with Šidák correction was performed to locate differences. Correlation between changes in MPS with feeding and leg fat mass were assessed by linear regression analysis. Statistical analysis was performed using the Prism v6.0 for Windows software (GraphPad Software Inc., USA). Data are reported as means ± SEM with significance accepted at the $P < 0.05$ level.

## Results

*Adipokine expression*

Subjects were recruited based upon both their body mass index and basal serum CRP concentration. As a consequence, serum CRP concentration was greater in the obese versus the healthy weight individuals ($P<0.001$; Table 1). This selection strategy was effective at recruiting obese individuals with chronic low-grade inflammation relative to their healthy weight study counterparts, as evidenced by the greater plasma concentrations of leptin, resistin, TNFα and IL-6 in the obese individuals. Conversely, plasma concentrations of adiponectin were significantly lower in obese versus healthy weight subjects (Table 1) as anticipated (10).

*Body composition, muscle strength and muscle fatigue*

As expected, BMI was greater in the obese than that of the healthy weight participants ($31.9 \pm 1.1$ and $23.4 \pm 0.3$ $kg.m^{-2}$, respectively; $P<0.001$). When examined in further detail using DXA, significantly greater fat masses were observed in the obese compared to the healthy weight subjects in all 5 body compartments interrogated (Figure 2A; $P<0.001$). Lean mass was also significantly greater in the obese individuals in the trunk (18% higher), android (26%) and gynoidal (14%) regions compared to the healthy weight subjects (Figure 2B). Lean tissue mass in the arms and legs of the lean and obese subjects was no different between the two subject groups.

There was no significant difference in the isometric strength of the knee extensors between the healthy weight and obese individuals ($34.3 \pm 2.5$ versus $33.9 \pm 3.0$ kg, respectively; Figure 3A). Similarly, the volume of work performed during 30 maximal isokinetic knee extensions

was equivalent between subject groups (Figure 3B), as was a measure of fatigue calculated from changes in torque output over time (Figure 3C).

*Gene expression analysis*

mRNA expression levels for 90 genes spanning inflammation, carbohydrate and fat metabolism, insulin signalling and proteolysis were examined. Using a false discovery rate (FDR) < 0.10, 7 transcripts appeared differentially expressed in obese compared to lean volunteers (Table 2). Cytochrome c, peroxisome proliferator activated receptor-α, PPAR-γ coactivator 1-α and transcription factor A mitochondrial, all associated with either mitochondrial biogenesis or the control of mitochondrial oxidative phosphorylation, were expressed at lower levels in the muscle of obese individuals. Similarly, CD34, a marker of satellite cell quiescence, and the solute carrier organic anion transporter family member 1B1, involved in hepatic drug metabolism, were both lower in the obese. In contrast, the expression of myostatin, which has been shown to be a negative regulator of muscle growth, was greater in obese skeletal muscle (1.80-fold compared to controls; $P<0.01$). These changes are consistent with a general deconditioning of muscle in the obese subjects relative to healthy weight volunteers. Interestingly, in spite of the systemic low-grade inflammation seen in the obese volunteers, muscle mRNA levels for TNFα and IL-6 were not elevated.

*Carbohydrate metabolism*

Serum insulin concentrations under clamp conditions are presented in Figure 4A. The insulin infusions rates of 0.6 and 15 $mU.m^{-2}.min^{-1}$ produced steady-state serum insulin concentrations of 5.2 ± 1.2 and 69.7 ± 5.4 $mU.L^{-1}$ in the lean, respectively, and 4.2 ± 0.3 and 78.7 ± 4.4 $mU.L^{-1}$ in the obese; there was no significant difference in either absolute serum insulin concentration or area under the curve during steady-state conditions between groups (Figure

4A). The 0.6 $mU.m^{-2}.min^{-1}$ insulin infusion resulted in negligible LGD in both subject groups (data not shown). In contrast, under steady-state conditions (210 to 240 min) the greater insulin infusion rate (15 $mU.m^{-2}.min^{-1}$) resulted in an increased rate of LGD in both sets of individuals, but was more marked in the lean (3.7 ± 0.4 $g.min^{-1}$) than that of the obese (1.3 ± 0.2 $g.min^{-1}$) subjects over the 30 min period examined (Figure 4B; $P<0.001$). The RER was no different between subject groups in the simulated post-absorptive state (0.72 ± 0.02 and 0.68 ± 0.01 in lean and obese subjects, respectively; Figure 4C). As expected, the simulated post-prandial state increased the RER in the healthy weight individuals (0.82 ± 0.03; $P<0.001$), but had no effect on obese volunteers where RER remained unchanged (0.71 ± 0.01).

*Muscle protein turnover*

During the 0.6 $mU.m^{-2}.min^{-1}$ insulin infusion when mixed-amino acids were not being provided, the rates of MPS, LPS and LPB were equivalent between the healthy weight and obese volunteers (Figure 5A, 5C and 5D). Under these conditions, the rate of LPB exceeded LPS and as such, net leg phenylalanine balance was negative but equivalent between groups (Figure 5E).

When serum insulin concentrations were increased and mixed-amino acids provided, arterial plasma phenylalanine concentrations doubled, from 60-80 μM to 130-150 μM in all volunteers (data not shown). Similarly, a doubling of the myofibrillar protein fractional synthetic rate was observed in healthy weight individuals (0.047 +/- 0.004 during fasted conditions versus 0.099 ± 0.011 $\%.h^{-1}$ under fed conditions; $P<0.001$), but no significant increase was observed in the obese volunteers (Figure 5A). When LPS was assessed by calculating phenylalanine disappearance into the leg (accepted to be a less sensitive approach

than muscle FSR), this difference between groups was still apparent but not significant (Figure 5C). While a main effect of hyperinsulinemia and hyperaminoacidemia to decrease LPB rates was observed, along with a trend towards an interaction between main effects (P=0.10; Figure 5D), proceeding with post-hoc tests revealed the decrease in LPB rates was confined to the obese (48.5 ± 9.5 fasted versus 29.9 ± 5.5 nmol.min$^{-1}$.100 g leg mass$^{-1}$ fed; P<0.01). Importantly, femoral blood flow, which impacts upon the determination of LPB rates, was equivalent between subject groups during both the fasted and fed clamps (Table 3). The culmination of these individual effects on LPS and LPB was that net leg phenylalanine balance was significantly enhanced in both subject groups (25.4 ± 6.7 versus 12.6 ± 5.1 nmol.min$^{-1}$.100 g leg mass$^{-1}$ in the lean and obese, respectively; Figure 5E). Comparison of leg fat mass with the net change in muscle protein synthesis rates in the simulated post-prandial state revealed a weak but significant (P=0.05) negative correlation between the two variables (Figure 5B).

*Anabolic signalling*

Under simulated post-absorptive conditions, total and phosphorylated protein levels of anabolic signalling intermediaries AKT and mTOR were of comparable magnitude between muscle samples of obese individuals and their healthy weight counterparts (Figure 5F). The simulated post-prandial state resulted in a significant and equivalent increase in AKT Thr$^{308}$ and mTOR Ser$^{2448}$ phosphorylation levels in both groups.

**Discussion**

Here we present novel evidence to suggest that obesity in non-frail, older men is not associated with deficits in lean mass, quadriceps strength or fatigability compared to healthy weight men of comparable age, despite systemic (but not muscle) inflammation being evident. Furthermore, we demonstrate for the first time that the ability of amino acids to increase MPS is blunted in obese, older men compared to their healthy weight counterparts, but that net leg phenylalanine balance is not affected due to a concomitant decrease in LPB in these individuals. In short, obesity appears to be associated with systemic inflammation, and altered MPS and LPB responses to increased nutrient delivery in older, non-frail men, but not reduced muscle mass or contractile function. Despite this, differences in whole body RER, LGD, and muscle mRNA changes consistent with a decline in overall muscle metabolic quality in obese older men were evident. These findings represent an important contribution to our understanding of the impact and interaction of systemic inflammation, ageing and obesity on muscle health.

In the present study the lean masses of all body regions examined were found to be equivalent or greater in obese men compared with healthy weight counterparts of similar age. Moreover, isometric strength, and work output and fatigability during repeated maximal isokinetic contractions were identical between groups. This stands in contrast to the suggestion that obesity in old age accelerates muscle mass loss and functional decline (7; 8). Our lack of evidence to support the existence of increased sarcopenia or dynapenia in older, non-frail, obese men is however perhaps not surprising. In young individuals, obesity is typically associated with a 36% greater lean mass compared to lean counterparts of similar stature (24), thought due to the additional contractile work performed by the obese individual during locomotion and daily living. Furthermore, recent evidence has shown dynapenia to occur only

in a subset of obese older adults with prevalence of the condition largely equivalent between lean (36%) and obese (27%) individuals (9), suggesting that dynapenia occurs independently of obesity. However, we acknowledge that our observations need to be confirmed in a larger cohort using more sensitive measures of lean tissue mass than DXA, such as MRI or D3-creatine dilution.

While previous studies have attempted to detail the effects of obesity on MPS in young adults, a consensus has not emerged. For example, obesity has been associated with lower (25) and elevated (26) MPS in the post-absorptive state. Furthermore, whilst greater rates of whole-body protein synthesis have been observed in obese younger volunteers in the fed state (27; 28), the magnitude of increase in MPS from the post-absorptive to simulated fed state was comparable between non-obese and obese volunteers (25). In contrast, we report here that under post-absorptive conditions where muscle FSR is at its lowest, the rate at which muscle proteins were being synthesised was comparable between healthy weight and obese older men. More importantly, the stimulatory effect of increased amino acid provision during hyperinsulinaemia on MPS in the older obese adult was blunted when compared to their healthy weight counterparts. The exact basis for this “anabolic resistance” to amino acid provision is unclear, but is unlikely to be related to muscle inflammation given we could find no evidence of this despite clear systemic inflammation. One potential contributor is the increased intracellular accumulation of lipids within the muscles of obese individuals. A negative correlation between leg fat mass and the degree of stimulation of leg protein synthesis was observed ($P<0.05$), suggesting that the resistance to the anabolic actions of amino acids within the muscle tissue was as a direct result of the increased fat mass. Moreover, it has recently been shown that acute intravenous administration of a lipid emulsion (intralipid, 100ml/h) results in the blunting of MPS during a hyperinsulinaemic

euglycaemic clamp concomitant to amino acid feeding (29). However, whether this is a direct effect of lipid species on the mechanisms responsible for MPS, or is mediated via changes in insulin sensitivity cannot be deduced from the studies performed to date.

The possible role of chronically reduced habitual physical activity levels in the anabolic resistance observed in the present study cannot be underestimated. Indeed, a recent study demonstrated that reducing daily step count by ~76% for 14 days in older individuals resulted in a 26% reduction in post-prandial rates of MPS and a 43% reduction in insulin sensitivity, but did not impact protein synthetic rates under post-absorptive conditions (30). Our own data demonstrate overt traits of muscle deconditioning were evident in the obese volunteers of the present study. For example, steady-state LGD and whole body carbohydrate oxidation rates were blunted in the obese volunteers, both of which are known to accompany inactivity (31). Furthermore, between group differences in muscle mRNA expression clearly indicates mitochondrial biogenesis and oxidative metabolism were dampened. Importantly, whilst daily physical activity levels were not measured in the present study, evidence suggests that even a 1 hr period of daily vigorous exercise cannot compensate for the effects of inactivity on blood markers of poor musculoskeletal health if the remainder of the day is spent sitting (32). These findings support the assertion that greater muscle deconditioning had occurred in the obese individuals in the present study.

Despite a failure of amino acids to stimulate MPS in the older obese men, the ability of insulin and amino acids to stimulate AKT and mTOR phosphorylation was unperturbed. A discord between AKT/mTOR signalling and MPS is not without precedent. Following stepwise increases in serum insulin concentration during conditions of hyperaminoacidemia in healthy, young volunteers, AKT phosphorylation paralleled the rise in insulin concentration

but was not matched by further increases in MPS and mTOR phosphorylation (20), suggesting AKT phosphorylation reflects insulin concentration rather than any measure of protein synthesis. Likewise, with its suggested role as an amino acid sensor, mTOR phosphorylation may reflect extracellular amino acid availability rather than commitment of the muscle cell to enhance MPS. As such, our results show clearly that the failure of amino acids to stimulate MPS in the older obese men is not due to an inability to phosphorylate AKT and mTOR.

Whole-body protein breakdown has been found to be inhibited less in the fed state in obese versus non-obese younger subjects (25). However, given muscle is reported to account for only 25% of whole-body proteolysis in the basal state (33), the implications of these findings remain unclear. Despite obese individuals being in a heightened systemic-inflammatory state in the present study, this did not translate into increased LPB under post-absorptive conditions. Indeed, our results suggest that the rate of LPB in the post-prandial state was lower in obese than healthy weight volunteers. Therefore, the inability of amino acids to stimulate MPS in the obese appeared largely offset by a concomitant decline in the rate of LPB, culminating in the magnitude of change in net phenylalanine balance between the post-absorptive and post-prandial state being equivalent between lean and obese subjects. This represents one potential mechanism for the equivalent leg lean mass seen between groups, although the consequence of assessing volunteers under acute conditions in the rested state is that the contribution of habitual physical activity levels and dietary behaviour on chronic muscle protein turnover and thereby muscle mass, remains unknown.

Obesity in older men is aligned with systemic, but not muscle, inflammation. We found no evidence that obese, non-frail, older men are at increased risk of accelerated muscle mass loss

or impaired contractile function (strength and fatigability) compared to their healthy weight counterparts. However, our results highlight the negative effect that obesity has on the metabolic quality of skeletal muscle in older adults. The exact role that inactivity plays in the decline in muscle metabolic health in the older obese adult remains unclear, but it could prove the central causative feature and should be the focus of future work.

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**Table 1: Subject characteristics**

| | **Lean (n=15)** | **Obese (n=11)** |
|---|---|---|
| Age (yrs) | 66.7 ± 1.1 | 66.4 ± 1.8 |
| Height (cm) | 176 ± 2 | 175 ± 2 |
| Weight (kg) | 72.8 ± 2.0 | 97.7 ± 3.6 *** |
| BMI ($kg.m^{-2}$) | 23.4 ± 0.3 | 31.9 ± 1.1 *** |
| Body fat mass (%) | 22.7 ± 1.5 | 36.4 ± 1.0 *** |
| Diastolic blood pressure (mmHg) | 83 ± 1 | 84 ± 2 |
| Systolic blood pressure (mmHg) | 131 ± 2 | 141 ± 2 ** |
| Total blood cholesterol concentration ($mmol.l^{-1}$) | 5.8 ± 0.3 (n=12) | 6.5 ± 0.4 (n=8) |
| Fasting blood glucose concentration ($mmol.l^{-1}$) | 4.68 ± 0.15 | 4.81 ± 0.19 |
| Fasting serum insulin concentration ($mU.l^{-1}$) | 5.2 ± 0.2 | 10.1 ± 1.8 ** |
| Serum c-reactive protein concentration ($\mu g.ml^{-1}$) | 0.69 ± 0.09 | 4.20 ± 0.83 *** |
| Plasma adiponectin concentration ($ng.ml^{-1}$) | 11.6 ± 1.1 | 6.7 ± 1.2 ** |
| Plasma IL-6 concentration ($pg.ml^{-1}$) | 0.84 ± 0.16 | 1.98 ± 0.29 ** |
| Plasma leptin concentration ($ng.ml^{-1}$) | 2.35 ± 0.31 | 9.58 ± 1.15 *** |

| | | |
|---|---|---|
| Plasma resistin concentration ($ng.ml^{-1}$) | 4.93 ± 0.34 | 6.43 ± 0.49 * |
| Plasma TNFα concentration ($pg.ml^{-1}$) | 1.21 ± 0.20 | 2.28 ± 0.47 * |
| Medications | Aspirin 75 mg daily (n=1) | Atenolol 50 mg daily (n=1); aspirin 75 mg daily (n=1); lisinopril 40 mg daily; germaloids cream as required (n=1). |

Values represent means ± SEM. *** P<0.001, ** P<0.01, * P<0.05 significantly different from lean subjects.

**Table 2: Changes in mRNA levels of several transcripts associated with metabolism or muscle growth in skeletal muscle of obese older men.**

| Pathway | Gene | Gene code | Probe identifier | Fold change | P-value | FDR |
|---|---|---|---|---|---|---|
| Apoptosis | Apoptotic peptidase activating factor 1 | APAF1 | Hs00559441 | 1.04 | 0.582 | 0.856 |
| | B-cell CLL/lymphoma 2 | BCL2 | Hs00608023 | 1.19 | 0.055 | 0.291 |
| | Caspase 8 | CASP8 | Hs01018151 | 1.04 | 0.727 | 0.894 |
| | Caspase 9 | CASP9 | Hs00154261 | 0.97 | 0.637 | 0.861 |
| | Diablo, IAP-binding mitochondrial protein | DIABLO | Hs00219876 | 0.98 | 0.693 | 0.890 |
| Autophagy | BCL2/adenovirus E1B 19kda interacting protein 3 | BNIP3 | Hs00969291 | 1.07 | 0.269 | 0.676 |
| | Dynamin 1-like | DNM1L | Hs00247147 | 0.91 | 0.027 | 0.223 |
| | GABA(A) receptor-associated protein | GABARAP | Hs00925899 | 0.88 | 0.028 | 0.223 |
| Calcium release | Ryanodine receptor 1 | RYR1 | Hs00166991 | 1.00 | 0.988 | 0.988 |
| | Ryanodine receptor 3 | RYR3 | Hs00168821 | 0.65 | 0.033 | 0.223 |
| Carbohydr | Creatine kinase | CKM | Hs00176490 | 0.21 | 0.411 | 0.839 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ate metabolism | Lactate dehydrogenase B | LDHB | Hs00929956 | 0.91 | 0.919 | 0.985 |
| | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 | NDUFA5 | Hs00606273 | 0.83 | 0.008 | 0.102 |
| | Pyruvate dehydrogenase kinase, isozyme 4 | PDK4 | Hs00176875 | 1.10 | 0.786 | 0.932 |
| | Solute carrier family 22 (organic cation/ergothioneine transporter), member 4 | GLUT4 | Hs00268200 | 0.89 | 0.814 | 0.953 |
| Inflammation | Interleukin 6 | IL-6 | Hs00985639 | 0.67 | 0.405 | 0.839 |
| | Metallothionein 1A | MT1A | Hs00831826 | 0.84 | 0.533 | 0.856 |
| | Tumor necrosis factor α | TNFα | Hs99999043 | 0.80 | 0.423 | 0.839 |
| Insulin signalling | Eukaryotic translation initiation factor 2B, subunit 2 | EIF2B2 | Hs00204540 | 0.84 | 0.015 | 0.162 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | beta | | | | | |
| | Mitogen-activated protein kinase 3 | MAPK3 | Hs00385075 | 0.90 | 0.06 | 0.306 |
| | Mitogen-activated protein kinase kinase 1 | MAP2K1 | Hs00605615 | 1.04 | 0.568 | 0.856 |
| | Phosphoinositide-3-kinase, regulatory subunit 1 (alpha) | PIK3R1 | Hs00933163 | 0.59 | 0.148 | 0.489 |
| | Protein kinase, AMP-activated, alpha 1 catalytic subunit | PRKAA1 | Hs01562308 | 0.94 | 0.141 | 0.482 |
| | Ras homolog family member Q | RHOQ | Hs00817629 | 1.07 | 0.183 | 0.550 |
| | RAS p21 protein activator (gtpase activating protein) 1 | RASA1 | Hs00243115 | 0.98 | 0.710 | 0.890 |
| | Ras-related GTP binding A | RRAGA | Hs00538975 | 1.01 | 0.779 | 0.932 |
| | V-akt murine thymoma viral oncogene homolog 2 | AKT2 | Hs01086102 | 0.95 | 0.275 | 0.676 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Lipid metabolism | Carnitine palmitoyltransferase 1B | CPT1B | Hs00993896 | 0.78 | 0.080 | 0.322 |
| | Carnitine transporter OCTN2 | SLC22A5 | Hs00161895 | 0.69 | 0.164 | 0.524 |
| | Farnesyl diphosphate synthase | FDPS | Hs00266635 | 0.95 | 0.666 | 0.876 |
| | Fatty acid binding protein 3 | FABP3 | Hs00269758 | 0.79 | 0.107 | 0.411 |
| | Fatty acid synthase | FASN | Hs00188012 | 1.12 | 0.489 | 0.856 |
| | Fatty acid translocase/CD36 | CD36 | Hs00169627 | 0.93 | 0.413 | 0.839 |
| | Heat shock 70kda protein 5 | HSPA5 | Hs99999174 | 1.04 | 0.575 | 0.856 |
| | NADH dehydrogenase (ubiquinone) Fe-S protein4 | NDUFS4 | Hs00942568 | 0.87 | 0.068 | 0.322 |
| | Protein kinase C, alpha, | PRKCA | Hs00925195 | 0.94 | 0.555 | 0.856 |
| | Protein kinase C, | PRKCE | Hs00178455 | 1.02 | 0.859 | 0.963 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | epsilon | | | | | |
| | Protein kinase C, iota | PRKCI | Hs00702254 | 1.06 | 0.548 | 0.856 |
| | Protein kinase C, theta | PRKCQ | Hs00989970 | 0.97 | 0.630 | 0.861 |
| | Stearoyl-coa desaturase 1 | SCD | Hs01682761 | 1.30 | 0.550 | 0.856 |
| Myogenesis | **CD34 molecule** | **CD34** | **Hs00990732** | **0.79** | **0.004** | **0.068** |
| | m-cadherin 15 | CDH15 | Hs00170504 | 1.09 | 0.292 | 0.700 |
| | Met proto-oncogene | MET | Hs01565584 | 1.13 | 0.191 | 0.556 |
| | Myogenic factor 5 | MYF5 | Hs00271574 | 1.21 | 0.029 | 0.223 |
| | Ras homolog enriched in brain | RHEB | Hs00950800 | 1.00 | 0.971 | 0.985 |
| | Ras homolog family member A | RHOA | Hs00236938 | 1.06 | 0.247 | 0.659 |
| | Ras homolog family member B | RHOB | Hs00269660 | 1.09 | 0.367 | 0.820 |
| | Rho-associated, coiled-coil containing protein kinase 1 | ROCK1 | Hs00178463 | 1.00 | 0.949 | 0.985 |
| | V-Ha-ras Harvey rat sarcoma viral oncogene homolog | HRAS | Hs00610483 | 0.99 | 0.863 | 0.963 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Organic anion transporter | **Solute carrier organic anion transporter family, member 1B1** | **SLCO1B1** | **Hs00272374** | **0.05** | **0.004** | **0.068** |
| Oxidative phosphorylation / Electronic Transport Chain | Coenzyme Q10 homolog B | COQ10B | Hs00257861 | 1.24 | 0.497 | 0.856 |
| | COX-I, Cytochrome C oxidase I | MT-CO1 | Hs02596864 | 0.81 | 0.031 | 0.223 |
| | COXII, Cytochrome C Oxidase2 | MT-CO2 | Hs00153133 | 0.71 | 0.472 | 0.856 |
| | **Cytochrome c** | **CYCS** | **Hs01588974** | **0.70** | **0.000** | **0.014** |
| | Cytochrome c oxidase subunit viia polypeptide 1 | COX7A1 | Hs00156989 | 0.85 | 0.074 | 0.322 |
| | Succinate dehydrogenase complex II | SDHA | Hs00188166 | 0.89 | 0.077 | 0.322 |
| | Ubiquinol-cytochrome c reductase, complex III | UQCRQ | Hs00416927 | 0.85 | 0.055 | 0.291 |
| Proteolysis | Calpain 1 | CAPN1 | Hs00559804 | 1.03 | 0.513 | 0.856 |
| | Calpain 2 | CAPN2 | Hs00965092 | 0.96 | 0.477 | 0.856 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Calpain 3, (p94) | CAPN3 | Hs00544975 | 1.03 | 0.593 | 0.856 |
| | Calpastatin | CAST | Hs00156280 | 1.01 | 0.943 | 0.985 |
| | Cathepsin L1 | CTSL1 | Hs00377632 | 0.92 | 0.201 | 0.568 |
| | F-box protein 32 | MAFbx | Hs01041408 | 1.02 | 0.840 | 0.963 |
| | Matrix metallopeptidase 2 | MMP2 | Hs01548727 | 1.06 | 0.595 | 0.856 |
| | Proteasome subunit, beta 1 | PSMB1 | Hs00427357 | 1.04 | 0.666 | 0.876 |
| | Proteasome subunit, beta 2 | PSMB2 | Hs01002946 | 1.03 | 0.604 | 0.856 |
| | Proteasome subunit, beta 5 | PSMB5 | Hs00605652 | 1.02 | 0.714 | 0.890 |
| | Tripartite motif containing 63, E3 ubiquitin protein ligase | MuRF1 | Hs00261590 | 0.85 | 0.310 | 0.726 |
| | Ubiquitin B | UBB | Hs00430290 | 1.00 | 0.975 | 0.985 |
| | Ubiquitin C | UBC | Hs00824723 | 1.06 | 0.548 | 0.856 |
| | Ubiquitin specific peptidase 19 | USP19 | Hs00324123 | 1.06 | 0.226 | 0.619 |
| Regulator of cell | **Myostatin** | **MSTN** | **Hs00976237** | **1.8** | **0.004** | **0.068** |

| | | | | | | |
|---|---|---|---|---|---|---|
| growth | | | | | | |
| Transcription factor | Activating transcription factor 3 | ATF3 | Hs00231069 | 0.89 | 0.606 | 0.856 |
| | Forkhead box O1 | FOXO1 | Hs00231106 | 1.01 | 0.959 | 0.985 |
| | Forkhead box O3 | FOXO3 | Hs00818121 | 0.96 | 0.704 | 0.890 |
| | Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta | IKBKB | Hs00233287 | 0.95 | 0.532 | 0.856 |
| | Jun proto-oncogene | JUN | Hs00277190 | 1.08 | 0.380 | 0.829 |
| | Myocyte enhancer factor 2C | MEF2C | Hs00231149 | 1.00 | 0.972 | 0.985 |
| | Myogenic differentiation 1 | MYOD1 | Hs00159528 | 1.01 | 0.924 | 0.985 |
| | Myogenin (myogenic factor 4) | MYOG | Hs00231167 | 1.19 | 0.046 | 0.286 |
| | Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 | NFKB1 | Hs00765730 | 1.12 | 0.048 | 0.286 |
| | Nuclear factor of kappa light | NFKB2 | Hs00174517 | 1.06 | 0.519 | 0.856 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | polypeptide gene enhancer in B-cells 2 (p49/p100) | | | | | |
| | **Peroxisome proliferator-activated receptor alpha** | **PPARA** | **Hs00231882** | **0.77** | **0.002** | **0.068** |
| | Peroxisome proliferator-activated receptor delta | PPARD | Hs00602622 | 0.94 | 0.273 | 0.676 |
| | Peroxisome proliferator-activated receptor gamma | PPARG | Hs01115513 | 0.85 | 0.081 | 0.322 |
| | **Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha** | **PPARGC1A** | **Hs01016724** | **0.67** | **0.002** | **0.068** |
| | Sterol regulatory element binding transcription factor 1 | SREBF1 | Hs01088691 | 1.11 | 0.428 | 0.839 |
| | Sterol regulatory element binding | SREBF2 | Hs01081778 | 1.02 | 0.785 | 0.932 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | transcription factor 2 | | | | | |
| | **Transcription factor A, mitochondrial** | **TFAM** | **Hs01082775** | **0.88** | **0.006** | **0.088** |
| | V-rel reticuloendotheliosis viral oncogene homolog A | RELA | Hs00153294 | 1.00 | 0.96 | 0.985 |

Seven genes, highlighted in bold and underlined, satisfied the criteria FDR < 0.1 and were considered differentially expressed. Fold changes expressed relative to lean subjects.

**Table 3: Femoral blood flow in healthy weight and obese volunteers as assessed by Doppler ultrasound at baseline and in the last 30 minutes of the fasted and fed clamps.**

| | **Lean (n=15)** | **Obese (n=11)** |
|---|---|---|
| Baseline | 385 ± 34 | 358 ± 30 |
| Fasted state insulin clamp (insulin 0.6 $mU.m^{-2}.min^{-1}$) | 512 ± 48 | 472 ± 56 |
| Fed state insulin clamp (insulin 15 $mU.m^{-2}.min^{-1}$ plus mixed amino acid infusion (10 $g.h^{-1}$) | 529 ± 45 | 450 ± 51 |

Results expressed in ml/min and values represent mean ± SEM. A significant main effect of insulin treatment was observed ($P<0.001$). No significant main effect of BMI stratification was observed or an interaction between main effects.

**Figure 1: Study protocol for the measurement of muscle protein synthesis and leg protein breakdown in the post-absorptive (0-120 min) and post-prandial (120-240 min) states.** For clarity, A-V blood sampling is not indicated, but occurred at regular intervals throughout the study period.

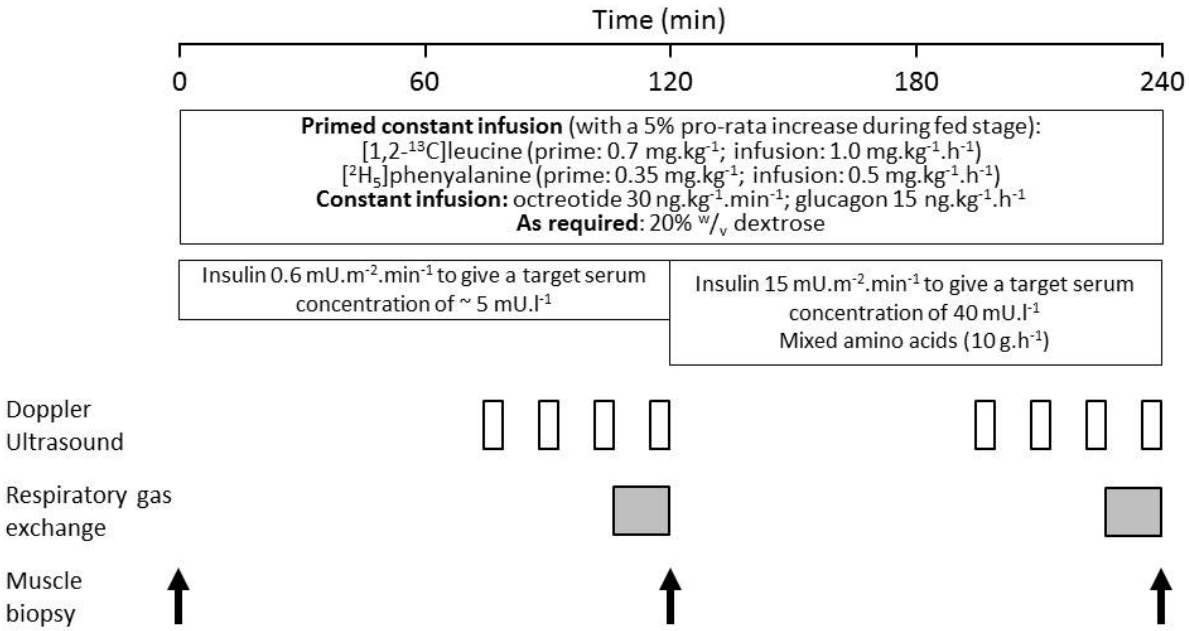


**Figure 2: Regional fat and lean masses in healthy weight and obese older men.** Mean values ± SEM for fat mass (A) and lean mass (B) in lean and obese volunteers separated by anatomical region. C) Diagrammatic representation of the approximate trunk, android and gynoid regions determined by the DXA imaging software. *** P<0.001, ** P<0.01, significantly different from healthy weight individuals.

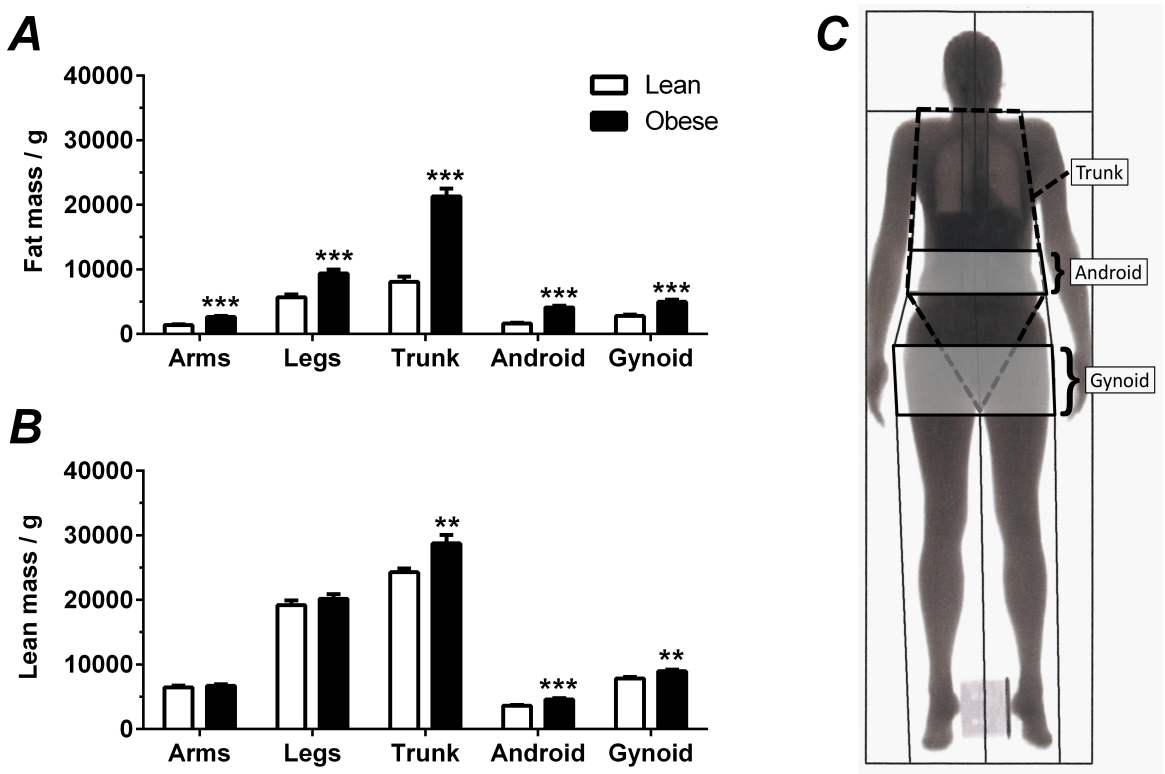


**Figure 3: Isometric strength, total work output and fatigue index during 30 maximal isokinetic knee extensions (at 90°.s$^{-1}$) in older healthy weight and obese men.** Values represent mean ± SEM for isometric strength (A), work done (B) and fatigue index ((peak torque-minimum torque)/peak torque) (C). No significant differences were observed between lean and obese individuals for any of the three parameters examined.

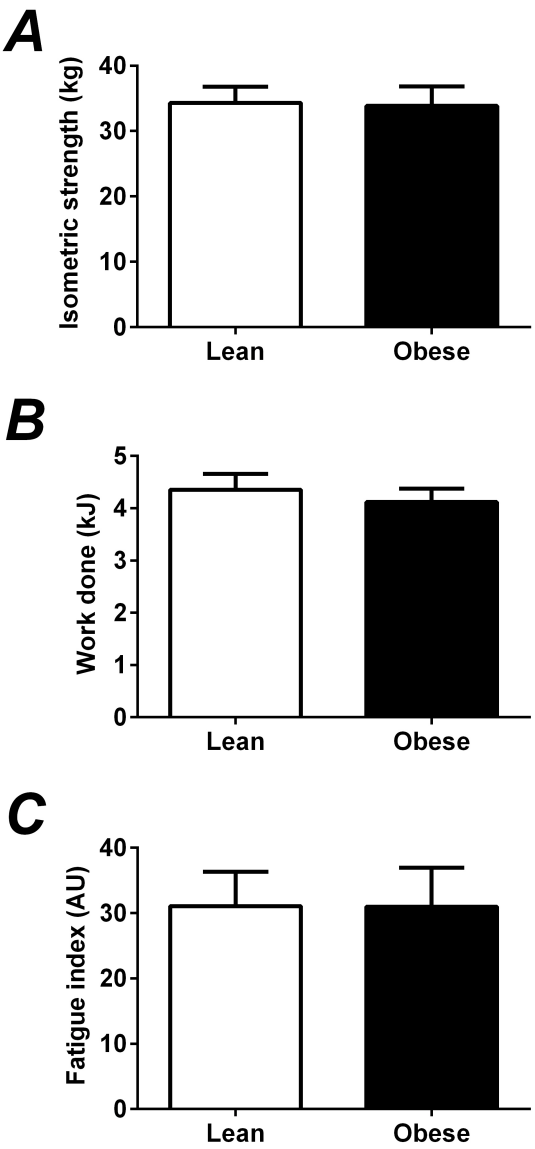


**Figure 4: Serum insulin concentration, leg glucose uptake and respiratory exchange ratio in fasted and fed state conditions in older healthy weight and obese men.** Mean ± SEM concentration of serum insulin (A) and leg glucose disposal (B) in response to a hypoaminoacidemia/hypoinsulinemic (0-120 min; insulin data only shown) clamp and a hyperaminoacidemia/hyperinsulinemic (120-240 min) euglycaemic clamp. Enclosed bar chart denotes area under the insulin curve (A) or leg glucose disposal rate (B) calculated over the last 30 min of the hyperaminoacidaemic hyperinsulinaemic clamp (shaded region on graphs A and B). Mean values ± SEM for respiratory exchange ratio (C) in healthy weight and obese older adults in both the post-absorptive and post-prandial states. Where relevant, P-values as determined by 2-way ANOVA for each main effect and interaction between main effects displayed alongside corresponding graph. NS: not significant; *** $P<0.001$, ** $P<0.01$ significantly different from healthy weight individuals; ††† $P<0.001$, significantly different from fasted clamp conditions.

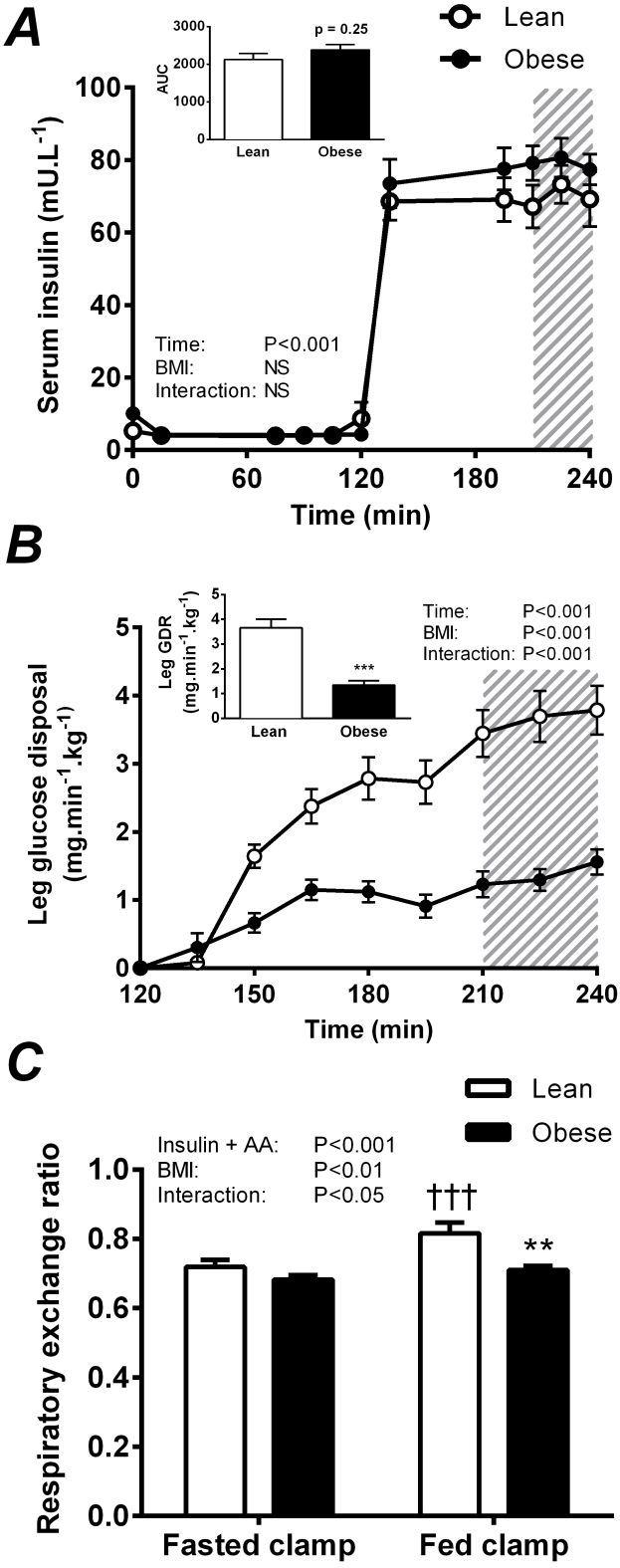
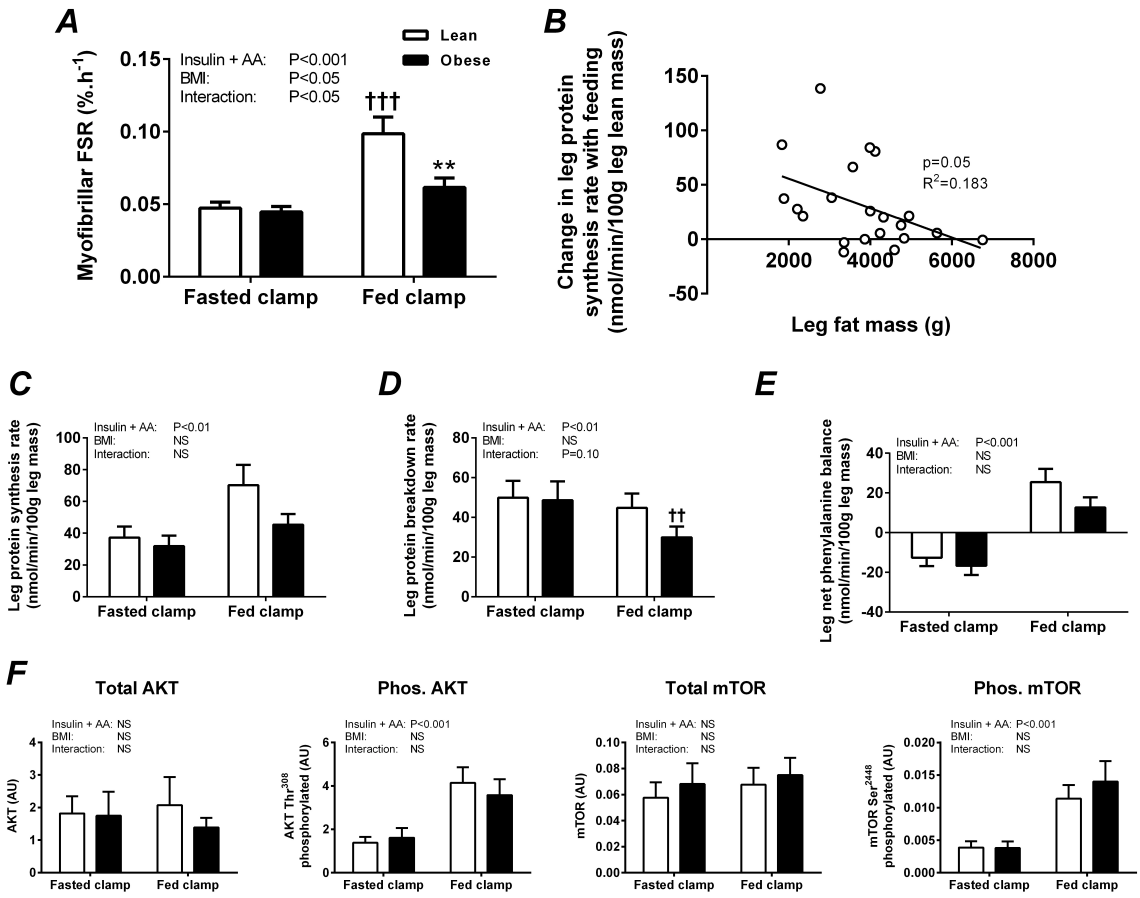

A
Lean
Obese
p = 0.25
AUC
3000
2000
1000
0
Lean
Obese
Serum insulin (mU.L-1)
100
80
60
40
20
0
Time: P<0.001
BMI: NS
Interaction: NS
0
60
120
180
240
Time (min)
B
Leg GDR (mg.min-1.kg-1)
5
4
3
2
1
0
***
Lean
Obese
Time: P<0.001
BMI: P<0.001
Interaction: P<0.001
Leg glucose disposal (mg.min-1.kg-1)
5
4
3
2
1
0
120
150
180
210
240
Time (min)
C
Lean
Obese
Insulin + AA: P<0.001
BMI: P<0.01
Interaction: P<0.05
Respiratory exchange ratio
1.0
0.8
0.6
0.4
0.2
0.0
†††
**
Fasted clamp
Fed clamp

**Figure 5: Muscle protein turnover and associated signalling in post-absorptive and post-prandial states in older healthy weight and obese men.** Myofibrillar fractional synthetic rate (A) assessed in healthy weight and obese individuals following simulated fasted or fed conditions. A negative correlation was observed between leg fat mass and change in muscle protein synthesis with feeding (B) which was significant by linear regression analysis (P=0.05). Leg protein synthesis rate (C), leg protein breakdown rate (D) and phenylalanine balance across the leg (E). To delineate the processes underpinning the observed changes in MPS, total protein levels and main phosphorylated forms of AKT and mTOR were determined by western blot (F). Bars represent mean values ± SEM. Where relevant, P-values as determined by 2-way ANOVA for each main effect and interaction between main effects displayed alongside corresponding graph. NS: not significant; ** P<0.01, significantly different from lean individuals; ††† P<0.001, †† P<0.01, significantly different from fasted clamp conditions.


A
Myofibrillar FSR (%.h-1)
Insulin + AA: P<0.001
BMI: P<0.05
Interaction: P<0.05
Lean
Obese
†††
**
Fasted clamp
Fed clamp
B
Change in leg protein synthesis rate with feeding (nmol/min/100g leg lean mass)
p=0.05
R2=0.183
Leg fat mass (g)
C
Leg protein synthesis rate (nmol/min/100g leg mass)
Insulin + AA: P<0.01
BMI: NS
Interaction: NS
D
Leg protein breakdown rate (nmol/min/100g leg mass)
Insulin + AA: P<0.01
BMI: NS
Interaction: P=0.10
††
E
Leg net phenylalanine balance (nmol/min/100g leg mass)
Insulin + AA: P<0.001
BMI: NS
Interaction: NS
F
Total AKT
Phos. AKT
Total mTOR
Phos. mTOR
AKT (AU)
AKT Thr308 phosphorylated (AU)
mTOR (AU)
mTOR Ser2448 phosphorylated (AU)