Habitual myofibrillar protein synthesis is normal in patients with upper GI cancer cachexia

Alisdair J MacDonald, Niel Johns, Nathan Stephens, Carolyn Greig, James A Ross, Alexandra C Small, Holger Husi, Kenneth C H Fearon, Tom Preston

1. Department of Clinical Surgery, University of Edinburgh, Edinburgh Royal Infirmary, 51 Little France Crescent, Edinburgh, EH16 4SA, UK
2. School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
3. Stable Isotope Biochemistry Laboratory, Scottish Universities Research Centre, East Kilbride, G75 0QF, UK
4. Institute of Cardiovascular and Medical Sciences, College of Medicine, Veterinary Medicine and Life Science, University of Glasgow, Glasgow G12 8QQ, UK

*Corresponding author: T Preston (Tom.Preston@glasgow.ac.uk)
Translational Relevance

Cachexia affects the majority of cancer patients with advanced disease and its treatment represents a major unmet clinical need. Muscle wasting is a key feature of the cachexia syndrome. Although studied in animal models, there are few studies in patients and none measuring muscle protein synthesis in free-living subjects at home. Here we have measured muscle protein synthesis in patients prior to undergoing surgery for upper GI cancer. We show that muscle protein synthesis is not decreased and may even be marginally increased. Our findings support the concept that a small increase in protein breakdown or altered myonuclear regeneration/loss may be the main mechanism accounting for muscle wasting in cancer patients. Our findings suggest that multimodal cachexia therapy should utilise anabolic strategies (since synthesis is not suppressed) and perhaps also target specific breakdown pathways/myonuclear regeneration to achieve maximum benefit.
Abstract (246 words)

Purpose: Skeletal muscle wasting and weight loss are characteristic features of cancer cachexia and contribute to impaired function, increased morbidity and poor tolerance of chemotherapy. This study used a novel technique to measure habitual myofibrillar protein synthesis in cancer patients compared with healthy controls.

Experimental design: An oral heavy water (87.5g deuterium oxide) tracer was administered as a single dose. Serum samples were taken over the subsequent week followed by a quadriceps muscle biopsy. Deuterium enrichment was measured in body water, serum alanine and alanine in the myofibrillar component of muscle using Gas Chromatography-Pyrolysis-Isotope Ratio Mass Spectrometry and the protein synthesis rate calculated from the rate of tracer incorporation. Net change in muscle mass over the preceding 3 months was calculated from serial CT scans and allowed estimation of protein breakdown.

Results: 7 healthy volunteers, 6 weight-stable and 7 weight-losing (≥5% weight loss) patients undergoing surgery for upper GI cancer were recruited. Serial CT scans were available in 10 patients, who lost skeletal muscle mass preoperatively at a rate of 5.6%/100d. Myofibrillar protein fractional synthetic rate was 0.058, 0.061 and 0.073 %/h in controls, weight-stable and weight-losing patients respectively. Weight-losing patients had higher synthetic rates than controls (p=0.03).

Conclusion: Contrary to previous studies, there was no evidence of suppression of myofibrillar protein synthesis in patients with cancer cachexia. Our finding implies a small increase in muscle breakdown may account for muscle wasting.
Introduction

Loss of skeletal muscle mass in cancer is well described (1-3) and is a defining characteristic of the cachexia syndrome (4). Low muscle mass is associated with a variety of clinical consequences including prolonged hospitalisation, reduced mobility and increased chemotherapy toxicity (5). A key objective of supportive cancer care is to preserve or increase muscle mass and perhaps improve outcomes during cancer treatment. In normal day to day living, muscle mass is generally thought to be maintained by the balance between muscle protein synthesis and breakdown. An alternative process is the balance between muscle regeneration via activation of satellite cells and myo-nuclear loss via apoptosis(6).

A reduction in muscle mass can occur due to an increase in muscle protein breakdown, a decrease in synthesis or a combination of the two and all variations have been described in animal models of cancer cachexia (7). However, there are many limitations when extrapolating such models to the human situation, including the more acute versus chronic timescales of tumour growth and development of muscle wasting (8). Due to the invasive nature of clinical protocols to date, there are few studies of skeletal muscle protein metabolism in human cancer cachexia and these have demonstrated either markedly reduced (9) or unchanged (2) FSR when compared with controls. In a previous study of patients with upper GI cancer cachexia, we have demonstrated that the transcriptome in human skeletal muscle demonstrates down regulation of ~1700 genes (mostly connected with cell turnover) and up-regulation of ~100 genes, none with known connections to protein degradation via the ubiquitin proteasome pathway (10). This led to the conclusion that reduced synthesis was the likely cause of muscle wasting in such patients.

Previous studies of skeletal muscle protein synthesis in humans have used an infusion protocol with a stable isotope labelled amino acid. Under controlled conditions in a clinical research facility such protocols are ideal for estimating the short term response (2 – 6 hours) to specific stimuli such as feeding or exercise, but are unsuitable for measuring skeletal muscle synthesis in individuals living in the community. An alternative method uses the oral administration of a deuterium tracer in the form
of heavy water (H$_2$O). With this method, endogenous or intrinsic labelling of dispensable amino-acids occurs and the latter become incorporated into muscle protein. The predictable kinetics and long elimination half-time of body water allows a period of one to two weeks between tracer administration and muscle biopsy (11-14). Thus the participant can return home, eat normally and resume normal activity during the study period. Using such methodology skeletal muscle protein synthesis has been measured in the elderly over a 6 week period with regular tracer doses and fractional synthetic rate (FSR) found to be 1.06 %/d or 0.044%/h (15). In young men (using a single dose over a 48 hour period) FSR was estimated at 1.45 %/day or 0.060 %/h (16). We have previously developed a protocol in healthy volunteers whereby muscle FSR was measured to be 0.058 %/h over a 1-2 week period after a single oral dose (17). The method is made possible by the use of Gas Chromatography-Pyrolysis-Isotope (GC-P-IRMS), allowing the measurement of the very low enrichments resulting from an economic dose of tracer. This single dose protocol is ideal for clinical use as it reduces the burden on the patient, it allows measurement of body composition and can be modified to include total energy expenditure. The present study aimed to measure habitual myofibrillar protein FSR in healthy volunteers versus weight-losing and weight-stable patients with cancer. Secondary aims were to compare quadriceps muscle FSR with rectus abdominis FSR and to examine the influence of muscle loss on muscle FSR.

Methods

Subjects/Protocol

The protocol received approval by the Lothian Research Ethics Committee. Written informed consent was obtained from all participants. All procedures were in accordance with International Conference on Harmonization Guidelines on Good Clinical Practise and the Helsinki Declaration. Healthy volunteers were screened using a simple health questionnaire (18), patients planned for upper gastrointestinal cancer surgery were recruited at the Royal Infirmary Edinburgh, UK. A medical history was recorded along with current height and weight and a self reported pre-illness weight from
which total weight loss was calculated. A single oral dose of 133g 70atom% of deuterium oxide was administered under supervision at the start of the protocol. Serial blood and urine samples were taken at baseline and 24 hours, 72 hours and on the final day of the study. For the healthy volunteers, a quadriceps biopsy was taken under local anaesthetic using a Bergstrom needle and subsequently snap frozen in liquid nitrogen. Combined quadriceps and rectus abdominis biopsies were taken under general anaesthesia at the start of surgery in the cancer patients.

Laboratory methods

The procedure for preparation and analysis of samples was performed as previously described (17). A brief outline is described below.

Serum samples

One ml of serum was used for analysis in triplicate of ²H enrichment in body water by continuous flow IRMS following equilibration with H₂ gas (19). One ml of each serum sample was deproteinsed by ultrafiltration prior to analysis of free amino acid enrichment. Plasma free amino acids were purified by cation exchange and prepared for GC-pyrolysis-IRMS analysis as ethoxy carbonyl ethyl esters (17). Basal deuterium abundance in protein-bound alanine from serum albumin was measured (20) and assumed to be equal to basal abundance in myofibrillar protein (21).

Processing of muscle biopsies

Rectus and quadriceps skeletal muscle biopsies were processed to isolate the myofibrillar component. Muscle biopsies of ~30µg were homogenised on ice and centrifuged at 10,000 x g for 15 minutes. The supernatant underwent serial Triton x-100 washes and centrifugation with the myofibrillar pellet being retained. Rectus abdominis muscle was additionally processed for recovery of free intracellular amino acids. After homogenisation and centrifugation the initial supernatant was processed for separation of free intracellular amino acids by ultrafiltration. Myofibrillar protein isolates (~2 mg) were subjected to gas phase acid hydrolysis (6 M HCl; 150°C for 4 hours) and the resulting amino acids were derivatised as ethoxy carbonyl ethyl esters prior to analysis of deuterium enrichment and the relative concentration of individual neutral amino acids by GC-pyrolysis-IRMS (17).
**GC-pyrolysis-IRMS of amino acids**

In basic outline, 4 µL aliquots of the volatile amino acid derivatives from protein hydrolysates and free amino acids were injected into the GC-pyrolysis-IRMS with the injector at 250°C and the column oven at 50°C. After a 1 minute hold, amino acids were separated on a 60m x 0.32 mm x 0.5 µm film DB-Wax column programmed to 150°C at 25°C/min, then to 220°C at 2.5°C/min and then at 7.5°C/min to 250°C, where it was held for 1 minute. Through the action of a microfluidics Deans switch, neutral amino acids were diverted on-line into a 1350°C capillary pyrolysis furnace to convert analytes to H₂ gas. The H₂ gas entered the IRMS ion source through an open/split separator. H₂ was ionised and hydrogen isotopes were separated and their abundance measured by comparison with a reference gas.

**Body composition**

Deuterium dilution space was calculated from the intercept of the elimination plot divided by the dose, following the methodology as used in the doubly-labelled water protocol (22). Total body water (TBW) was calculated from deuterium space assuming non-aqueous hydrogen exchange factor of 1.041. Fat free mass was calculated from TBW by assuming FFM hydration was 73.2% (23). Skeletal muscle mass (SMM; kg) was estimated from FFM using the MRI-validated equations of Wang (24) which describe the ratio of SMM:FFM with gender and age. A myofibrillar protein content of 12.4% SMM was assumed (25).

**CT measures of body composition**

Cross sectional measures of muscularity were taken from routine CT scans in patients in the pre-operative period according to the previously described method (26). Muscle CSA was measured with SliceOmatic V4.3 software (Tomovision, Canada). In order to provide clinical context and to allow comparison with total body water measures of body composition, cross sectional measurements were converted to whole body estimates of FFM using the formula of Mourtzakis et al (26). Muscle mass
values were adjusted for height and expressed as cm\(^2\)/m\(^2\). Low muscularity was defined as a skeletal muscle index measured from CT <55.5 cm\(^2\)/m\(^2\) for men and 38.9 cm\(^2\)/m\(^2\) for women (1). Myosteatosis was defined as mean HU<41 for BMI<25 or HU<33 for BMI≥25 (3). Where a single time-point CT scan was used this was the immediate pre-op scan. Fractional breakdown rate (FBR) was estimated from measured CT net muscle loss and FSR assuming, FBR = FSR – rate of loss. Whole body estimates of myofibrillar synthesis and breakdown were made assuming that quadriceps FSR is similar to skeletal muscle FSR in all muscles.

Statistics

Statistical analysis was performed using SPSS v21. Group data are presented as median and range. Group comparisons were made using either Mann-Whitney U test or Wilcoxon Signed Ranks Test for paired data. Comparison across multiple groups was performed using the Jonckheere-Terpstra trend test for non-parametric data where the groups have order (27, 28). Correlations were tested by Spearman’s rho.

Results

Group characteristics

Seven healthy volunteers and 14 patients with ‘resectable’ upper GI cancer were recruited. There were 9 patients with oesophageal cancer (pT1-3, N0-2) and 5 patients with gastric cancer (pT1-4, N0-2). 11/15 patients had undergone pre-operative neo-adjuvant chemotherapy. All patients were studied at least 4 weeks after their last dose of chemotherapy. The patient group was stratified into weight-stable and weight-losing groups using weight loss ≥5%. Group characteristics are described in Table 1. Groups were well matched with the exception of age in the weight-losing cancer group who were slightly older.
Validation of plasma alanine enrichment

In order to validate the use of plasma alanine enrichment as a proxy for intracellular free alanine (the immediate precursor of alanyl-t-RNA), 1cm³ rectus abdominis muscle biopsies were obtained from a sub-group of the cancer cohort (n=11). Free intracellular alanine enrichment was compared with the respective enrichment in serum samples taken at the start of surgery. Unless otherwise stated, tracer enrichment quoted here is as analysed, that is, the ethoxy carbonyl ethyl alanine ester has 15 hydrogen atoms of which up to 4 may become labelled. The enrichment of the 4 hydrogen atoms that may become labelled via body water is thus greater by a factor of 15/4. However, as every hydrogen atom is converted to H₂ gas, the enrichment as measured is given. Median enrichment was 366 ppm ²H excess (range: 305-455) in free intracellular alanine from rectus and 325 ppm ²H excess (range: 225-450; the wide range is a function of a different fat free mass and different labelling time of each biopsy) in serum. Intracellular alanine and serum alanine enrichment were correlated (r² 0.73, p=0.01, Spearman’s rho). The analytical variance was considerably greater in the intracellular free pool samples due to the small volume of the intracellular sample.

Total body water enrichment was measured in serum and urine samples. We confirmed our earlier findings that ²H enrichment of body water predicts that of serum free alanine with an average of 3.64 H atoms in alanine becoming labelled (17). In the current study, a total of 43 pairs of urine and serum samples were available across all patients and time-points. ²H₂O enrichment in urine and serum was correlated significantly (r² 0.998 p<0.0001), a finding which validates the use of urinary ²H₂O enrichment as a proxy for precursor enrichment in future studies.

Skeletal muscle myofibrillar protein FSR

When patients were categorised by weight loss, there was a trend in quadriceps myofibrillar protein FSR across all three groups (Healthy controls < weight stable patients with cancer < weight losing patients with cancer) (p=0.022). Quadriceps FSR was higher in weight-losing patients with cancer (0.073 % per hour) compared with healthy volunteers (0.058% per hour) (Figure 1) (p=0.03).
Quadriceps myofibrillar protein FSR was 0.058% per hour in healthy volunteers compared with 0.067% per hour in all patients with cancer (p=0.079).

Comparison of quadriceps and rectus FSR within weight-loss categories revealed no difference between quadriceps and rectus in the weight-stable group (p=0.34) but significantly higher in quadriceps versus rectus in the weight-losing patients (p=0.021). Post-hoc comparisons revealed no significant associations (Spearman’s rho) between quadriceps FSR or rectus FSR with skeletal muscle index (p=0.322, p=0.537), mean Hounsfield units (p=0.583, p=0.625), or the presence of myosteatosis (p=0.544, p=0.856). Low muscularity was not associated with altered quadriceps FSR (p=0.463) low muscularity quadriceps FSR=0.063 %/hr range 0.043-0.082, normal muscularity FSR=0.067 %/hr, range 0.058-0.074). However, rectus FSR was greater in low muscularity patients (FSR=0.063 %/hr, range 0.042-0.069) than normal muscularity patients (FSR=0.046 %/hr, range 0.036-0.061) (p=0.038, Mann-Whitney U test).

Baseline body composition and pre-op loss of skeletal muscle mass

CT scans were undertaken immediately prior to surgery for all 14 patients with cancer. A measure of skeletal muscle cross sectional area at the level of the third lumbar vertebrae was available for each. Of these 14 patients, 9 were classified with low muscularity and 5 were classified as myosteatotic.

Sequential scans were available in 10 patients with a mean interval time of 79 days. Summary baseline (i.e. before neoadjuvant chemotherapy) body composition measures are shown in Table 2. Over the subsequent 79 days, all but one patient lost muscle mass. Median muscle loss was 5.6%/100d. There was no correlation between muscle loss and muscle FSR using either quadriceps or rectus measures (p=0.531, p=0.940).

Myofibrillar protein synthesis and breakdown

Skeletal muscle FBR was not different between weight-stable (n=3, FBR -0.056%hr, range -0.049 to -0.067) and weight-losing patients with cancer (n=7, FBR -0.061, range -0.037 to -0.075) (p=0.833).

Skeletal muscle mass estimated from deuterium dilution in only those cancer patients who underwent serial CT scans is given in Table 3. Loss of muscle mass as revealed by CT scans enabled FBR data to
be calculated in the patients in comparison with the control subjects, who were assumed to have stable muscle mass (thus, FSR=FBR). These early stage cancer patients showed similar muscle mass to controls and similar level of relative (FSR) and absolute myofibrillar protein synthesis (Table 3). In the cancer patients, myofibrillar protein breakdown was just 1.2 g/day (2.9%) greater than synthesis.

**Discussion**

The present study found that habitual skeletal muscle protein FSR tended to be higher in weight-losing versus either weight-stable cancer patients or healthy controls. These findings contradict the hypothesis that muscle wasting in cancer cachexia is caused by a reduction in protein synthesis. The present study is the first to measure skeletal muscle protein FSR during a period of days/weeks that incorporates the physiological variations associated with feeding/fasting and activity/rest. The finding supports our previous studies in similar patients with early stage (resectable) upper GI cancer that had shown normal physical activity levels (29) and relative preservation of protein intake (30) exercise and amino acids being two of the key stimuli for protein synthesis in skeletal muscle.

Two previous short term studies (<6 hours) using a primed constant infusion of isotope tracer have shown that skeletal muscle protein FSR may be reduced in the fed state in patients with lung cancer (9) or may be unchanged (colorectal cancer) but with a blunted response to intravenous infusion of amino acids (2). Any potential reduction in muscle protein FSR would be expected to be more apparent during the present protocol than during a short term infusion protocol as the oral tracer design incorporates the potentially cumulative anabolic effect of many meals over the one week protocol. As a result, there appears to be a genuine contradiction between the results of the current study and those of the previous infusion tracer studies.
In previous studies, we have demonstrated that in patients with upper GI cancer cachexia there is predominant downregulation of the transcriptome in human muscle consistent with a reduction in cell turnover/protein synthesis (10). The present study demonstrates a potential disconnect between regulatory pathways in the transcriptome and the gross level of protein synthesis in the myofibre. Studies in small animals have suggested a central role for AKt (Protein Kinase B) signalling and the regulation of skeletal muscle protein synthesis. However, when considered in humans, there appears to be a dissociation between cell signalling (AKT) and myofibrillar protein synthesis during feeding, exercise and immobilisation (31). No investigation of the cellular mechanisms of protein synthesis or degradation were performed during this study so it is only possible to speculate on possible explanations for the differences found between groups. One possible mechanism to account for such divergent findings is the presence of an alternative pathway sensitive to acute physiological change/damage such as regenerative myogenesis. Indeed, muscle mass may be influenced by the balance between myofibre loss (apoptosis/autophagy) and myocyte regeneration from satellite cells. In muscle biopsies from weight-losing patients with upper gastro-intestinal cancer we have shown a threefold increase in muscle DNA fragmentation compared with control subjects. The increase in DNA laddering was associated with an increase in poly(ADP-ribose) polymerase (PARP) cleavage, both observations being consistent with enhanced apoptosis (32). Concerning muscle regeneration, He and co-workers (33) have recently described in both tumour-bearing mice and patients with pancreatic cancer, activation of both satellite and non-satellite muscle progenitor cells. However, there appeared to be a downstream block to regeneration via NFkB-mediated Pax7 dysregulation and this was thought to contribute to net muscle atrophy.

In a recent study Williams and co-workers (3) assessed myofibrillar FSR in both the fasting and post-absorptive state. The expected increase of FSR with feeding was observed in the control subjects but was lost in patients with cancer. This finding suggests that muscle wasting in cancer cachexia could occur due to a blockade to normal anabolic stimuli and highlights the importance of considering the
intermittent effects of normal stimuli to protein synthesis when estimating the overall impact of cachexia on muscle synthesis over time.

An alternative explanation for the different findings between the present and previous studies may depend on the nature of the anabolic response to feeding or exercise. Conventionally FSR may be considered at a basal rate representing un-stimulated muscle protein turnover with periodic peaks occurring in response to feeding or exercise. In cachexia, an anabolic block could result in a simple reduction in FSR during feeding, resulting in a reduction in habitual FSR. Alternatively, instead of a reduced FSR peak with feeding, the response may be blunted and/or prolonged, possibly as the result of delayed clearance of dietary amino acids from the splanchnic bed (34).

In the present study the median muscle loss was 5.6% per 100 days prior to surgery. In the group of 10 cancer patients with serial CT measurements, median body weight was 71 kg and median muscle mass was 20.2 kg (Table 3). In the cancer patients, median myofibrillar protein synthesised per day was 41.2 g (Table 3). Median myofibrillar protein breakdown was estimated to be 42.4 g/day, or 1.2g/day (2.9 %) greater than synthesis. In the context of whole body protein synthesis, myofibrillar protein may account for 20-25%, with mixed muscle protein contributing 33-40% of total body protein synthesis. The imbalance between breakdown and synthesis of only 2.9% is perhaps the most important observation. Such a small, but vitally important imbalance between synthesis and breakdown is difficult to quantify. Accurate assessment of the situation necessitates long term measures of myofibrillar protein synthesis along with serial measurements of muscle mass on an individual basis. Only then will it be established if these observations can be generalised and if they relate to the disease stage studied.

One explanation of loss of skeletal muscle could be due to modestly increased degradation. Direct measures of skeletal muscle degradation using arterio-venous difference methodology has shown increased degradation in patients with cancer compared with controls (2). Skeletal muscle synthetic and degradative pathways are closely dependent. Conventionally this interdependence is understood to reduce synthesis when degradation is increased and vice versa (35). However, this interaction appears to be more complex, even in health where increased muscle breakdown is seen in response to
the anabolic stimulus of resistance or endurance exercise (36). As such, it is possible that the changes seen in cachectic muscle reflect a process of deranged skeletal muscle remodelling where synthesis is necessarily increased but counter-acted by increased muscle degradation. Alternatively the increased synthesis could be the result of an inadequate compensatory mechanism to balance pathological muscle breakdown. In mice, increased skeletal muscle FSR in the presence of net loss of muscle protein occurs during denervation induced muscle wasting (37-41). The mechanism for an increase in muscle synthesis during net protein loss could be through stimulation of protein synthesis via mTORC1. This protein complex, normally associated with the maintenance of muscle mass and muscle hypertrophy could be activated by the increase in amino acids released as a result of increased protein breakdown by the proteasome (41). In a murine model of cancer cachexia, increased levels of phosphorylated Akt and increased expression IGF-1 in muscle of tumour bearing AH-130 and C26 mice suggests that muscle wasting in tumour bearing animals may not be associated with down regulation of molecules involved in the anabolic response (42). In further support of a counter-regulatory response to compensate for muscle loss in cachexia, Op den Kamp and co-workers (43) have recently observed increased Akt phosphorylation in the muscle of cachectic lung cancer patients. However, downstream phosphosubstrates glycogen synthase kinase 3β, MTORC and Forkhead box protein were unaltered and this may be evidence for overall impaired anabolic signalling. Furthermore, these complex interactions may alter with disease stage.

There are a number of potential limitations to the present study. First, the number of patients and volunteers was relatively small and matching treatment effects, diet and exercise was not possible. The small numbers resulted in groups that were not matched exactly for age (i.e. the patient group was older than the volunteer group). However, it is unlikely that a difference between the groups is masked as older individuals are likely to have a reduced FSR or reduced response to anabolic stimuli (44, 45). The patient group had undergone neoadjuvant chemotherapy. The regimens used (either cisplatin and 5 Fluorouriacil or epirubacin, cisplatin and capecitabine) are not known to increase protein synthesis and have previously been associated with loss of muscle mass (46) and so are unlikely to confound the results. No comparison of nutritional intake was made between patient and
volunteer groups. The possible use of nutritional supplements in the patient group could act as a stimulus to protein synthesis and the lack of an accurate record of nutritional intake represents one potential limitation of this study. With the exception of one patient who was dependent on nasogastric feeding, no patient was prescribed or reported taking oral nutritional supplements during the study period. Secondly, the present study used a novel protocol for the measurement of skeletal muscle FSR and it was not known at the outset whether the method would have sufficient sensitivity to distinguish between small groups. However, recent studies in healthy individuals have demonstrated an increase in myofibrillar FSR with resistance exercise in young individuals (14) and our observations reported here show a significant 11% difference in myofibrillar protein FSR between quadriceps and rectus muscles. Third, a degree of bias may arise from contamination of the myofibrillar sample from labelled amino acids from non-myofibrillar sources such as collagen or sarcoplasmic proteins. Care was taken during this protocol to ensure no such contamination occurred. The myofibrillar isolation protocol was chosen to ensure repeated washing of the myofibrils to eliminate sarcoplasmic contamination. An additional advantage of the long protocol duration was the low precursor enrichment which results in reduced risk of contamination of the myofibrillar fraction with labelled free amino acids. Contamination with collagen was equally unlikely; this was demonstrated by the absence of high concentrations of glycine and proline in the protein hydrolysates.

In the present study, different rates of myofibrillar protein FSR were observed in the quadriceps femoris versus the rectus abdominis muscles. This finding is consistent with previous direct measures of FSR in different muscle groups where triceps FSR differed from quadriceps FSR by ~15% in healthy individuals (47). The magnitude of the difference in FSR between quadriceps and rectus is, however, small and the clinical significance of the finding is uncertain. It does, however, demonstrate the importance of comparing the same muscle between subject groups and that the technique can detect small differences in FSR.

In conclusion, myofibrillar skeletal muscle FSR measured in free living individuals over the period of one week is not reduced in patients with cancer compared with healthy volunteers. These findings
support the potential value of multimodal rehabilitation programmes for cancer patients that include anabolic strategies such as exercise or nutritional support to help prevent cachexia.

Acknowledgements

We thank the patient and volunteer participants, Kathryn Sangster, Jim Black and the staff of the Welcome Trust Clinical Research Facility. We are also grateful for the support of the Melville Trust and an ESPEN research fellowship award.

Legends

Tables

Table 1 – Descriptive characteristics of groups by weight loss category with comparison of control participants vs weight stable patients with cancer and controls vs weight losing patients with cancer

Table 2 – Baseline body composition measures derived from CT in all patients with cancer (n=14).
Table 3 – Skeletal muscle mass (kg), absolute myofibrillar protein synthesis and absolute myofibrillar protein breakdown (g/day). Cancer patients with serial CT scans are included (n=10; comprising 7 weight losing and 3 weight stable subjects). Muscle mass was estimated at the time of consuming deuterium oxide. Control subjects (n=7) were assumed to have stable muscle mass. Protein kinetics were derived from quadriceps biopsy data.

Figures

Figure 1 – Category scatterplot of quadriceps myofibrillar protein FSR (%/hr) for healthy controls, weight stable patients and weight losing cancer patients. Horizontal line marks median value.

Figure 2 – Waterfall chart of Pre-operative skeletal muscle loss by patient as measured on cross sectional area from sequential CT scans (during pre-operative neo-adjuvant chemotherapy) (hashed bars = weight stable patients, open bars = weight losing patients). The median interval between scans was 79 days.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>IQR</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>52.1</td>
<td>51.5-53.1</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.4</td>
<td>67.1-80.8</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68</td>
<td>1.65-1.71</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5</td>
<td>24.7-26.4</td>
<td></td>
</tr>
<tr>
<td>Sex M:F</td>
<td>3:4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-stable cancer</td>
<td>62.5</td>
<td>57.0-70.3</td>
<td>0.051</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.3</td>
<td>78.0-93.3</td>
<td>0.073</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78</td>
<td>1.74-1.78</td>
<td>0.101</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2</td>
<td>25.7-29.8</td>
<td>0.295</td>
</tr>
<tr>
<td>Sex M:F</td>
<td>4:2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-losing cancer</td>
<td>63.4</td>
<td>61.5-66.3</td>
<td>0.009</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.0</td>
<td>62.9-72.3</td>
<td>0.463</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7</td>
<td>1.65-1.75</td>
<td>0.613</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5</td>
<td>20.9-26.2</td>
<td>0.397</td>
</tr>
<tr>
<td>Sex M:F</td>
<td>4:4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight loss (%)</td>
<td>0</td>
<td>0-0</td>
<td></td>
</tr>
</tbody>
</table>

*Weight stable or weight losing versus control (Mann-Whitney U).

Table 2

<table>
<thead>
<tr>
<th>Body composition measures</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle mass index (cm²/m²)</td>
<td>41.7</td>
<td>39.3-47.7</td>
</tr>
<tr>
<td>Mean HU</td>
<td>36.3</td>
<td>32.3-38.8</td>
</tr>
<tr>
<td>CT-derived Fat Free Mass (kg)</td>
<td>44.8</td>
<td>39.8-51.2</td>
</tr>
<tr>
<td>CT-derived Fat Mass</td>
<td>24.6</td>
<td>19.6-27.7</td>
</tr>
<tr>
<td>Fat Free Mass (kg; deuterium dilution)</td>
<td>46.3</td>
<td>41.9-49.8</td>
</tr>
<tr>
<td>Fat Mass (kg; deuterium dilution)</td>
<td>28.6</td>
<td>24.9-31.4</td>
</tr>
</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th></th>
<th>Cancer (n=10)</th>
<th>Control (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>71.0</td>
<td>66.8 - 80</td>
</tr>
<tr>
<td>Skeletal muscle mass (kg)</td>
<td>20.2</td>
<td>18.6 - 22.5</td>
</tr>
<tr>
<td>Myofibrillar protein synthesis (g/day)</td>
<td>41.1</td>
<td>38.2 - 41.8</td>
</tr>
<tr>
<td>Myofibrillar protein breakdown (g/day)</td>
<td>42.4</td>
<td>39.1 - 42.8</td>
</tr>
</tbody>
</table>
References


44. Proctor DN, Balagopal P, Nair KS. Age-related sarcopenia in humans is associated with reduced synthetic rates of specific muscle proteins. J Nutr. 1998;128(2 Suppl):351S-5S.


*P=0.03 weight losing cancer patients versus controls (Mann Whitney U)
Figure 2
Habitual myofibrillar protein synthesis is normal in patients with upper GI cancer cachexia


*Clin Cancer Res* Published OnlineFirst November 4, 2014.

**Updated version**
Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-14-2004

**Author Manuscript**
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.