ORIGINAL ARTICLE

Body composition skeletal muscle analysis in cancer cachexia studies: Is there a place for 3T MRI analysis?

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Abstract

Aims Cancer cachexia is a condition often seen in end stage Non-Small Cell Lung Cancer (NSCLC) patients. Recent developments include the use of pharmaceutical agents and/or exercise to induce stability/hypertrophy of muscle volume. This requires accurate assessment of the change in both quantity and quality of the muscle during cancer cachexia clinical studies. Magnetic Resonance Imaging (MRI) is appropriately placed to address both of these factors. The present study aimed to investigate total quadriceps muscle volume change by 3T MRI within a cancer cachexia clinical study.

Methods and results **A**uckland's **C**ancer **C**achexia **e**valuating **R**esistance **T**raining (**ACCeRT)** study is a randomised controlled feasibility study investigating eicosapentaenoic acid (EPA) and cyclo-oxygenase-2 (COX-2) inhibitor (celecoxib) (Arm A) versus EPA, COX-2 inhibitor (celecoxib), Progressive Resistance Training (PRT) plus essential amino acids (EAAs) high in leucine (Arm B) in NSCLC cachectic patients. All participants underwent 3T MRI scanning at baseline and at last or end of trial (EOT) visit. Analysis showed a mean total quadriceps muscle volume percentage change from baseline to EOT of +12.5% (Arm A), compared with -3% (Arm B). There was a difference in muscle volume between genders. Arm B participant data showed a percentage change of +4.2% within females (n=2) compared with -10.2% (n=2) within males at EOT visit. All EOT results suggests the use of EPA and celecoxib +/- PRT and EAAs could potentially preserve muscle volume loss during refractory cachexia.

Conclusions ACCeRT is the first study to utilise 3T MRI total quadriceps muscle volume within a cancer cachexia study, along with the first in an end-stage/refractory cachexia population. These results can be used for baseline/reference for future cancer cachexia studies targeting the anabolic muscle pathways in end-stage/refractory cachexia patients.

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Introduction

Cancer cachexia has recently been defined as a 'multifactorial syndrome defined by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment' (1), and now incorporates the development of the stages of cachexia including pre-cachexia, cachexia and refractory cachexia (1). Increased knowledge has been gained regarding the molecular pathways involved in the muscle wasting process, and it is hypothesised that muscle loss is the main component related to fatigue, and function impairment within cancer cachexia (2).

Over the last few decades, a number of pharmacological agents and methods of support have been investigated to address cancer cachexia. This has resulted in the use of various methods in determining one of the main clinical endpoints of body composition, including skeletal muscle mass throughout different cancer cachexia clinical studies.

Earlier studies utilised skin fold calculations, then later Bioelectrical Impedance Analyser (BIA) and more recently Dual-Energy X-ray Absorptiometry (DEXA) analysis and L3-CT (Lumbar-3 Computed Tomography) which is now considered the 'gold-standard' of Lean Body Mass (LBM) analysis (3).

BIA has been demonstrated to show good short-term precision in terms of test-retest reliability in patients with advanced cancer (4). Updated models encompassing multi-frequency BIA correlate closely with DEXA and provide a superior assessment when compared to single-frequency BIA (5). BIA utilises resistance and reactance to estimate total body water, and is

dependent on population-gained equations. Advantages of BIA include: non-invasive, high reproducibility and being less expensive than CT, DEXA, and Magnetic Resonance Imaging (MRI) acquisition and analysis. Limitations include: results can be confounded by ascites and lymphoedema depending on each cancer cohort. Other confounding factors include the possibility of dehydration in advanced cancer patients, which may lead to an underestimate of Fat Free Mass (FFM) (6, 7).

There has also been a change in the presentation of body composition data over time. Earlier studies presented data from BIA in terms of total body weight, Fat Mass (FM), and FFM. The latter is defined as total body mass minus all fat i.e. storage fat mass and essential fat mass. All the following cancer cachexia studies utilised BIA for body composition analysis; Madeddu *et al.* utilised BIA 101, (Akern Spa) within a phase II study which investigated an oral amino acid supplementation (8), and Del Fabbro *et al.* utilised Tanita TBF-310, (Tokyo, Japan) within a randomised, placebo-controlled study investigating melatonin (9).

There was then a change to using DEXA scans and reporting total body Lean Body Mass (LBM), defined as total body mass minus storage fat only, resulting in slightly higher values for LBM when compared to FFM. All the following cancer cachexia studies utilised DEXA for body composition analysis; Bayliss *et al*. within a series of clinical studies investigating clazakizumab (formally ALD518) (10), Macciò *et al*. within the phase III, randomised controlled study investigating a combined treatment within gynaecological cancer cachexia patients (11), and Dobs *et al.* within a phase II, randomised, placebo-controlled study investigating enobosarm (12).

DEXA utilises the principle of photon x-ray attenuation by different human tissues. Using a three-compartment model of bone mineral content/mass, FM and LBM, appendicular skeletal muscle mass can be determined. Advantages include low cost and low radiation exposure, with disadvantages including the low precision when compared to either MRI or CT and the lack of distinction between visceral and subcutaneous adipose tissue (6).

There has also been a change in analysing the data over time, as shown in the following studies. Temel *et al*. utilised DEXA for body composition within two phase III, randomised, placebo-controlled studies investigating anamorelin within ROMANA 1 and 2. Change in LBM defined as the average of change from baseline to week 6, and the change from baseline to week 12. This was considered by the study team to be a more conservative approach than just utilising change at week 12 data (13). More recently, data has been assessed as per change in kg/4 weeks as per the phase II, randomised, placebo-controlled study which investigated espindolol within the ACT-ONE study (14).

Choosing the incorrect modality has been shown to result in an imprecise and insensitive analysis and may hide positive and negative results, as seen in the following two studies. Mantovani *et al.* utilised three models for analysis of body composition within the phase III, randomised, five-arm study of different combination treatments. Results showed a non-significant change in terms of BIA LBM within all arms. When reviewing the different modalities, there was a non-significant change in mean BIA LBM analysis (p=0.609). However, data showed a significant change by DEXA analysis (p=0.0148) and a significant change in estimated LBM by L3-CT (p=0.001) for twenty-five participants all allocated to Arm 5 of the study (15). Madeddu *et al.* also utilised three models for analysis within the phase III randomised study of combination treatments. Results showed a non-significant change in terms of BIA LBM within both groups. Interestingly, a significant difference was seen within both groups in terms of LBM by both DEXA and L3-CT (16).

As seen above there has been a range of modalities investigating and analysing body composition within cancer cachexia clinical studies. This results in difficulty of comparisons of efficacy of either FFM, LBM, FM or total body weight between cancer cachexia studies.

Currently both CT and MRI are considered the gold standard in measurement of body composition (6). L3-CT single-slice taken at the 3rd lumbar vertebrae is strongly related to whole-body FFM and appendicular skeletal muscle mass as determined by DEXA. Advantages include reproducible results and high accuracy, with disadvantages including the relative cost of acquisition of scans and analysis, radiation exposure, and limited relevance to functional muscle groups (6, 17).

MRI is able to quantify the three adipose tissue depots: visceral, intramuscular and subcutaneous. Advantages include the lack of radiation exposure when compared to DEXA and CT imaging, and superior spatial resolution. Disadvantages include costs, such as the expense of acquisition, maintenance, technologist time, as well as analysis of the acquired data. Contraindications to MRI also need to be considered, which can exclude some patients from analysis by this modality (3, 6, 18).

Recent knowledge has been gained around the loss of skeletal muscle mass being the main component of cancer cachexia. This has led to the need to measure and quantify skeletal muscle, in terms of stabilisation or increase/loss in both skeletal muscle mass/volume and strength. Muscle strength and function can be inferred from the analysis of muscle volume, and measuring this over time is important in assessing changes during ageing, training and disease processes. The current 'gold standard' of measuring muscle volume involves utilising contiguous transverse MRI scans (19, 20).

Additional benefits of MRI include the analysis of both muscle volume and cross-sectional area (CSA), along with morphologic features and distribution. MRI can characterise the loss of muscle quality, e.g. intra-muscular fat infiltration, fibrous connective tissue and oedema (7, 21, 22). This is becoming important as loss of mobility has been shown to be related to muscle strength and increased muscle lipid content, which can

be quantified by both MRI and magnetic resonance spectroscopy (6, 7).

This study is the first to utilise data acquired by a 3T MRI scanner within a cancer cachectic study.

Materials and Methods

Auckland's **C**ancer **C**achexia **e**valuating **R**esistance **T**raining (**ACCeRT)** study is a single-centre, open-label, randomised controlled feasibility study. Participants were randomised in a 1:2 ratio into one of the following two treatment arms: **A)** EPA and COX-2 inhibitor (international best supportive care) or **B)** EPA, COX-2 inhibitor and PRT (2 sessions per week) plus 20 g EAAs high in leucine (treatment group). The study planned for 21 participants to be enrolled, for a study period of 20 weeks. No treatment arm crossover was permitted during the study. All participants completing the 20 week study, irrespective of which arm they were randomised to, were offered to continue or receive study medication/training sessions under compassionate use. Full study protocol published by Rogers *et al.* (23). Primary endpoint was the acceptability of a multi-targeted regimen in Non-Small Cell Lung Cancer (NSCLC) cachectic population, by completing a Likert based questionnaire. One of the secondary outcomes was the comparison between the two groups for total quadriceps muscle volume, using data gained from the 3T MRI analysis.

Participants

All participants enrolled on the above ACCeRT study attended the Centre for Advanced Magnetic Resonance Imaging (CAMRI), The University of Auckland, at Baseline and Last or End of Trial (EOT)/week 20 visit. All scans were acquired by personnel 'blinded' to treatment allocation. Please note, identical thigh for MRI scanning was used as for the leg strength testing (additional secondary endpoint); right thigh was preferred unless there was a contraindication.

Skeletal muscle volume measurements

Acquisition

VIBE transverse T1 weighted data was utilised in the analysis. MRI acquisition of the right/left thigh was performed as described (21). Briefly, MRI was performed in the supine position on the 3T Siemens Skyra scanner using two 18-channel phased array coils, and spine coils for signal reception. Slice thickness was set at 3 mm, 88 slices, and two contiguous acquisitions were obtained. The two scans were then stitched together for analysis.

Data were acquired with the following parameters: slice thickness 3 mm (no gaps); acquisition matrix 288 x 288; (interpolated to 576), field of view 250 mm; echo time (TE) 2.46ms; repetition time (TR) 5.73ms; and flip angle 10°. Two signal averages were obtained to improve the signal-to-noise ratio.

Analysis protocol

All scans were anonymised and coded for randomisation. Both observers were 'blinded' to the identifying parameters such as the subject's name and clinical data. A standardised analysis protocol was developed and implemented. This included; firstly identifying key anatomical landmarks, inferior aspect of the ischial tuberosity to the border of the patella, thereby defining the start slice and end slice number for each scan. The total quadriceps muscle volume (cm^3) was measured utilising the automated 'ITK-SNAP' segmentation software (24). Briefly, using the Active Contour Segmentation Mode, a region of interest was centred over the anterior compartment of the thigh. Quadriceps muscle was defined as the combined total volume of the vastus lateralis, vastus intermedius, vastus medialis and rectus femoris. Sartorius, adductor longus, short head of biceps femoris and the tensor of the fascia lata were excluded. An automated region growing algorithm was then used to coarsely segment the quadriceps muscle (Fig1a, b, c). Subsequently, each slice was individually analysed and refined using manual segmentation to ensure accuracy. When all scans were analysed, pairs of scans were then reviewed to confirm that anatomical landmarks and number of slices were similar, verifying the pre/post analysis.

Intra-observer and inter-observer agreement

Observer one (WO) carried out all 30 analyses, with observer two (ESR) carrying out three analyses (10%) for inter-observer rating. WO also carried out three repeat analyses one month later for intra-observer rating.

Fig1a, Images of full 3D segmented scan analysis

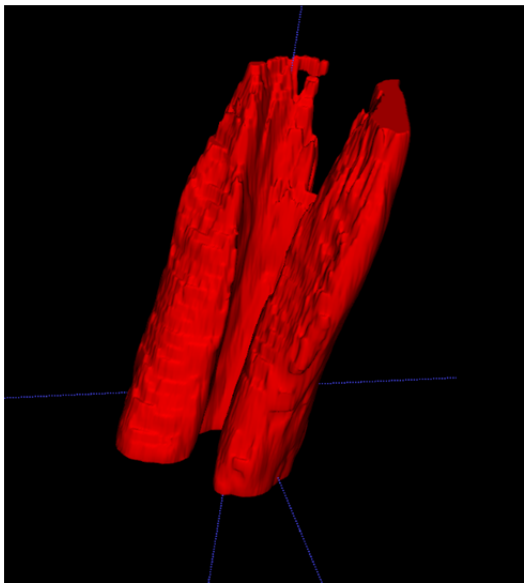


Fig1b, Images of full 3D segmented scan analysis

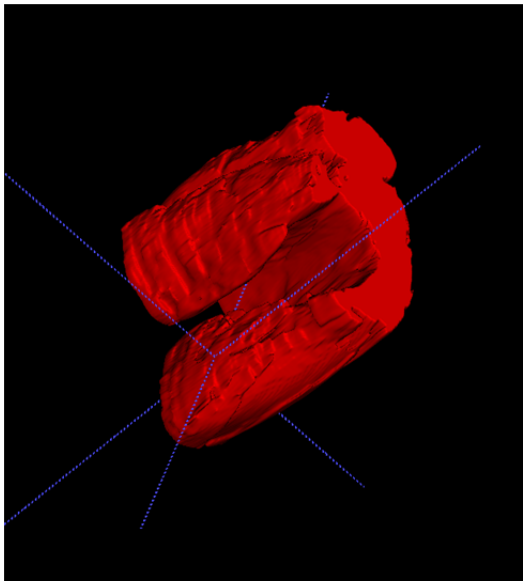
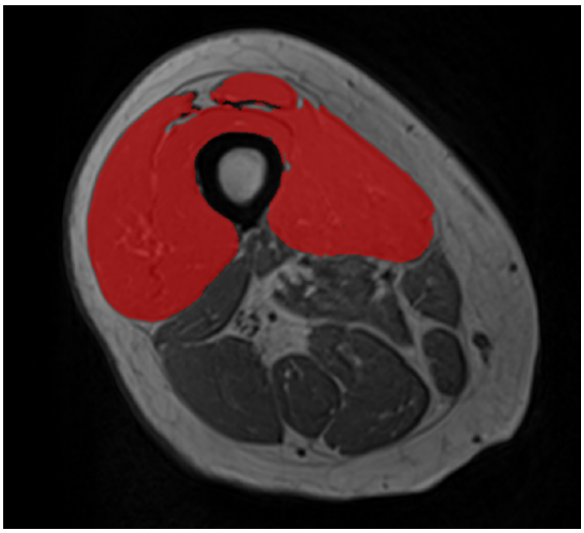


Fig1c Image of a single slice segmented scan analysis



Results

Patients

Seven participants were allocated onto Arm A, five males: two females. Thirteen participants were allocated onto Arm B, eight males: five females. All participants underwent a 3T MRI scan at baseline except one Arm B participant who had a cochlear implant and therefore was unable to undergo this study assessment. One Arm A participant underwent the scan but had images that were unable to be analysed because of difficulty differentiating adipose tissue from muscle. There was no objective difference in the signal intensities between the muscle and surrounding tissue, resulting in an inability to automatically segment and assess the volumes.

Due to the attrition of participants, pre and post treatment scan data was limited to two participants allocated to Arm A, and eight allocated to Arm B. In total thirty scans were available and analysed. Results presented with mean and range when number of participants >2, otherwise actual values presented if <2 participants. The baseline mean of all participants' MRI total quadriceps muscle volume showed a value of 925 cm^3 (range 669 to 1353 cm^3, n=6) within Arm A, compared with 890 cm^3 (range 562 to 1361 cm^3, n=12) within Arm B. These results indicate that at baseline, the range of MRI total quadriceps muscle volume was similar in both groups but the mean was slightly lower within Arm B, with a difference of 35 cm^3 between groups. There was a difference in volumes between genders. The baseline mean value for all females was 730 cm^3 (range 596 to 1011 cm^3, n=7), and for males, 1011 cm^3 (range 562 to 1361 cm^3, n=11) (Table 1).

Table 1 ACCeRT MRI total quadriceps muscle volume analysis at baseline

Baseline total quadriceps muscle volume (cm^3)			
	Total n=18 (male=11, female=7)	**Arm A n=6 (male=4, female=2)**	**Arm B n=12 (male=7, female=5)**
All	902 (562 to 1361)	925 (669 to 1353)	890 (562 to 1361)
Male	1011 (562 to 1361)	968 (820 to 1353)	1036 (562 to 1361)
Female	730 (596 to 1011)	840 (669 to 1011)	685 (596 to 771)

Table 2 ACCeRT MRI total quadriceps muscle volume percentage change from baseline to EOT visit

Total quadriceps muscle volume percentage change from baseline to week 20			
	Total n=6 (male=4, female=2)	**Arm A n=2 (male=2)**	**Arm B n=4 (male=2, female=2)**
All	+2.2 (-18.3 to +20.8)	+12.5 (+4.3, +20.7)	-3.0 (-18.3 to +4.8)
Male	+1.2 (-18.3 to +20.6)	+12.5 (+4.3, +20.7)	-10.2 (-18.3, -2.0)
Female	+4.2 (+3.6 to +4.8)		+4.2 (+3.6, +4.8)

Data shows the mean MRI total quadriceps muscle volume percentage change from baseline to EOT visit of +12.5% (+4.3%, +20.7%) within Arm A, compared with -3% (range -18.3 to +4.8%, n=4) within Arm B. These results indicate, on average, a net gain of total quadriceps muscle volume for participants within Arm A, compared with a net loss within Arm B. When analysing outcomes between genders, the net percentage change of +4.2% within all females (n=2) compared with +1.2% within all males (n=4) was seen at the EOT visit. When comparing the exercise (PRT) Arm B participant data, this showed the net percentage change of +4.2% within females (n=2) compared with net percentage change of -10.2% (n=2) within males at EOT visit. Non-exercise Arm A participant data showed the net percentage change of +12.5% (n=2) within both males at EOT visit (Table 2). Both of these Arm A participants experienced weight loss over the longest time period and were possibly at an earlier stage in the end-stage/refractory cachexia period. However, these results suggest that the use of EPA and celecoxib could potentially preserve muscle volume during this early refractory cachexia stage. The four participants within Arm B who completed the 20 week study had a mean percentage muscle volume loss of -3%. These data suggest that the use of EPA and celecoxib plus PRT and EAAs could potentially preserve muscle volume loss during the refractory cachexia stage.

Discussion

It was decided to utilise images gained from a 3T MRI scanner due to the clinical benefits compared to a 1.5T scanner. The 3T scanner has a stronger field strength that results in the increased signal-to-noise ratio, which from a physics basis is twofold. Images are clearer and allow super high resolution studies (1024 x 1024) to be undertaken within a quicker time frame (25, 26). The improved signal allows higher resolution and to cut thinner sections e.g. 2 mm thick, and musculoskeletal studies have improved fat saturation and higher resolution (25). Wong *et al.* retrospectively investigated visualisation of anatomical structures and image quality of scans acquired by 1.5T and 3T MRI scanners in patients undergoing scans of the knee. It was concluded that images from a 3T scanner provided improved diagnostic confidence and improved visualisation of anatomical structures by four independent radiologists (26). Chu *et al.* compared whole brain volumes gained by both 1.5T and 3T scanners in healthy subjects and patients with multiple sclerosis (27). Results showed that 1.5T volumes were generally higher when compared with 3T volumes. This was hypothesised to be due to a likely overestimation of whole brain volume within the 1.5T analysis, whereas 3T acquisitions had improved tissue contrast and definition, thereby increasing the accuracy and overall volume measurement (27). This suggests that it is difficult to compare and correlate 1.5T and 3T MRI scanner data in terms of visualising anatomical structures, boundaries and overall tissue volumes.

Greig *et al.* published results from the only other study investigating cancer cachexia utilising MRI scan data. Participants received the long acting β2-agonist formoterol to reverse muscle atrophy, and megestrol acetate to increase appetite. This study used a

1.5T MRI scanner with the following parameters. T1-weighted spin echo axial images were prescribed from the proximal border of the patella to the superior anterior iliac spine. Imaging parameters were: slice thickness 10 mm (no gaps); acquisition matrix 512 x 512; field of view 500 mm; echo time (TE) 15 ms; repetition time (TR) 425 ms; and flip angle 90°. Three signal averages were obtained to improve the signal-to-noise ratio. The number of slices acquired ranged from 38 to 45 depending on thigh length (28). This study had set a pre-defined definition of response; moderate response = 2.1 to 4% increased change, minor response = 0 to 2% increased change, minor progression = 0.1 to 2% decreased change, moderate progression = 2.1 to 4% decreased change and major progression = >4.1% decreased change (28). If taking these definitions for the ACCeRT study, individual data within Arm A shows two major responses with the net change of +4.3% and +20.7%, both over 20 weeks.

Within Arm B, there was one major and one minor responder with a net change of +4.8% and +3.6% respectively, and two non-responses with -2% and -18.3% over 20 weeks. Within Arm B, there were two non-responders/major progression of -25.6% and -15.4% at week 9 (Table 3) and of -7.9% and -21.4% at week 12 (Table 4).

When comparing data from the study by Greig *et al.* only seven participants completed the 8 week study. MRI data from both the left and right limbs, with range of change of -13.6% to +8.5% (mean +4%) for the right limb and range of -11.5% to +13.5% (mean +6%) for the left limb (28). These results are higher when compared to the above ACCeRT results, however participants were allowed to continue with concomitant chemotherapy during the study which could have affected the results, and/or the data was acquired on a 1.5T MRI scanner and data analysis could potentially have been overestimated as previously discussed.

As stated by Gray *et al.* scans of muscle from participants experiencing both cachexia and sarcopenia show non-contractile tissue within the muscle. The MRI analysis did show this to be present within the ACCeRT study populations as seen in Fig2. Greig *et al.* used k-means clustering to reduce this error (22, 28). It was not possible to omit the intramuscular adipose tissue from the overall analysis as the automated segmentation was not able to accurately and reliably differentiate it from the muscle at the pixel level. The use of an imaging protocol that selectively eliminates signal from these tissues during acquisition, such as a Dixon protocol, will help to overcome this issue in future studies.

The use of MRI total quadriceps muscle volume and cross-sectional areas have been used in other exercise intervention studies. A study investigating the exercise intervention in untrained women participating in endurance and strength training, gained imaging by a 1.5T MRI scanner at baseline and week 12 (20). MRI analysis segmented extensor, flexor, adductor and sartorius muscles. Participants were randomised to a supervised strength training (ST) group versus endurance training (ET) group versus a control group. ST and ET involved three sessions per week for 60 minutes. Over the 12 weeks a significant increase was seen within all muscles for participants allocated to the ST group with combined volume change of +14.5%, compared with only a significant increase in the sartorius and extensor muscles within the ET group, with a combined volume change of +10.4%. There were no relevant changes seen within the control group with a combined volume net loss of -2.8%. This study indicates the benefits of strength training over endurance training, along with the sensitivity of MRI scans to determine exercise-induced changes within the muscle, further supporting its use within future cancer cachexia clinical studies (20).

Results show (Table 5) the average number of slices scanned was 98, which took approximately 61 minutes for each segmented analysis. Interestingly, an abstract published from the eighth International Conference on Cachexia, Sarcopenia and Muscle Wasting, in Paris, France during December 2015, discussed a fast segmentation software that integrated the efficient interactive Random Walker segmentation algorithm into a convenient graphical user interface. A comparison was made between observers who performed a manual segmentation of the quadriceps volumes that required more than 5 hours, with observers producing similar volumes in less than 10 minutes with the presented software tool, thereby cutting down the above analysis time. However, most centres are already utilising a semi-automated software for muscle analysis and currently this study is awaiting full publication (29).

Both the MRI total quadriceps muscle volume data and BIA FFM data correlated well with correlation of 0.845 (Fig3) at baseline and of 0.828 (Fig4) at Last or EOT visit. This supports the trends seen with the BIA results within the main study.

It has been recently discussed the importance and rationale behind the choice of endpoints and that these should be compatible with the study intervention and/or action of drug (30). The ACCeRT study was investigating the acceptability of a multi-targeted approach encompassing a PRT element in this population and to quantify if this intervention had an effect on muscle volume and strength. By utilising 3T MRI analysis, this potential endpoint was correctly assessed. However, the ACT-ONE study which investigated the use of espindolol an anabolic/catabolic transforming agent, that increases anabolism through partial β-2 receptor agonism and reduces catabolism through non-selective β-blockade, utilised change in weight assessed by standard digital scale and DEXA scan analysis (14). Both POWER 1 and 2 studies which investigated enobosarm at multiple doses, again utilised the primary endpoint of LBM by DEXA and improvement in physical function by stair climb test (31). Currently there are twenty-three open clinical studies investigating cancer cachexia, with only one study (BAT-Cachexia study NCT02500004) utilising PET/MRI for body composition analysis (32).

Data has shown the benefit of exercise in attenuating intramuscular adipose infiltration routinely seen in the aging population, and that this reduction could be related to increased muscle quality and function in terms of relative strength and muscle power, even in the presence of stable muscle mass (33). Ideally new clinical studies targeting the muscle could assess both the muscle quantity and quality by MRI analysis.

The use of panoramic ultrasound (US) has been recently assessed in participants undergoing 70 days bed-rest. Results showed high accuracy in detecting simple changes in size in terms of hypertrophy and atrophy. This method also has the benefit of assessing muscle quality in terms of intramuscular adipose tissue and fibrous tissue within the muscle by echogenicity. It has not yet been utilised within a defined patient population or a cancer cachexia study (17).

Table 3 ACCeRT MRI total quadriceps muscle volume percentage change from baseline for participants ending at week 9

Total quadriceps muscle volume percentage change from baseline to week 9			
	Total n=2 (male=2)	**Arm A n=0**	**Arm B n=2 (male=2)**
All	-20.5 (-25.6, -15.4)		-20.5 (-25.6, -15.4)
Male			-20.5 (-25.6, -15.4)
Female			

Table 4 ACCeRT MRI total quadriceps muscle volume percentage change from baseline for participants ending at week 12

Total quadriceps muscle volume percentage change from baseline to week 12			
	Total n=2 (male=1, female=1)	**Arm A n=0**	**Arm B n=2 (male=1, female=1)**
All	-14.7 (-21.4 to -7.9)		-14.7 (-21.4, -7.9)
Male			-21.4
Female			-7.9

Fig2 Image of scan showing fatty infiltration

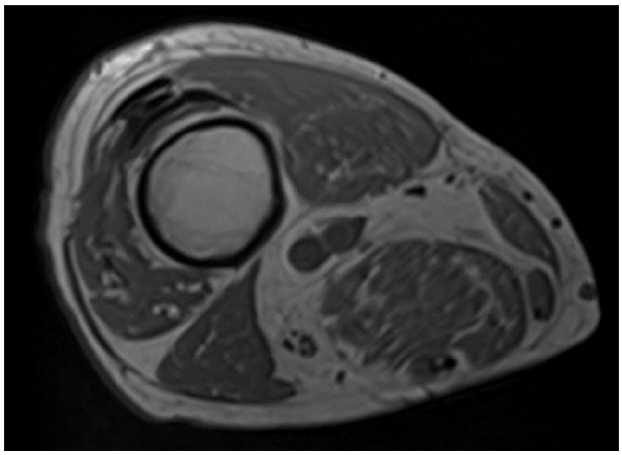
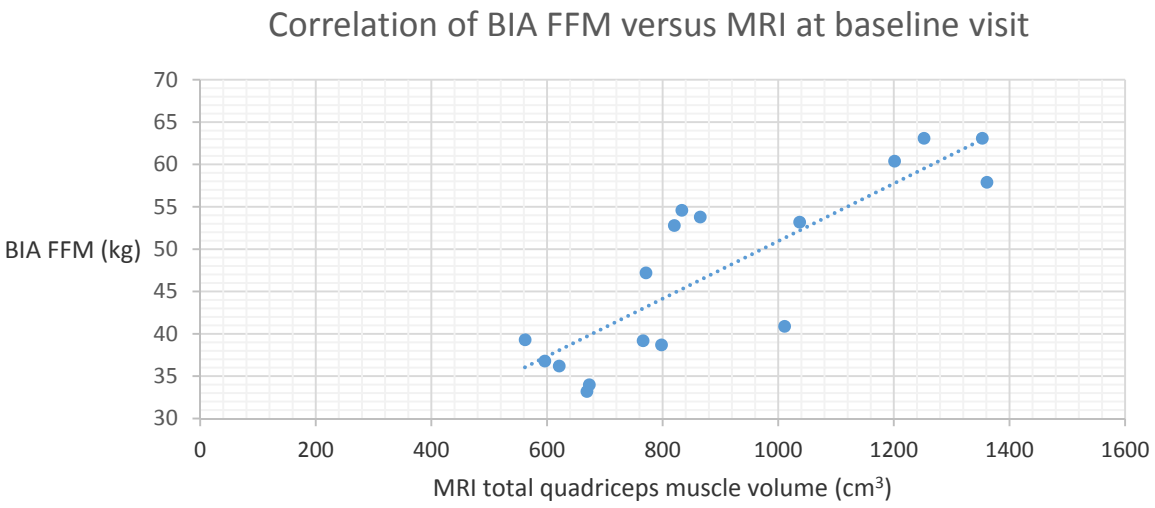


Table 5 Average slice number and time of analysis per scan

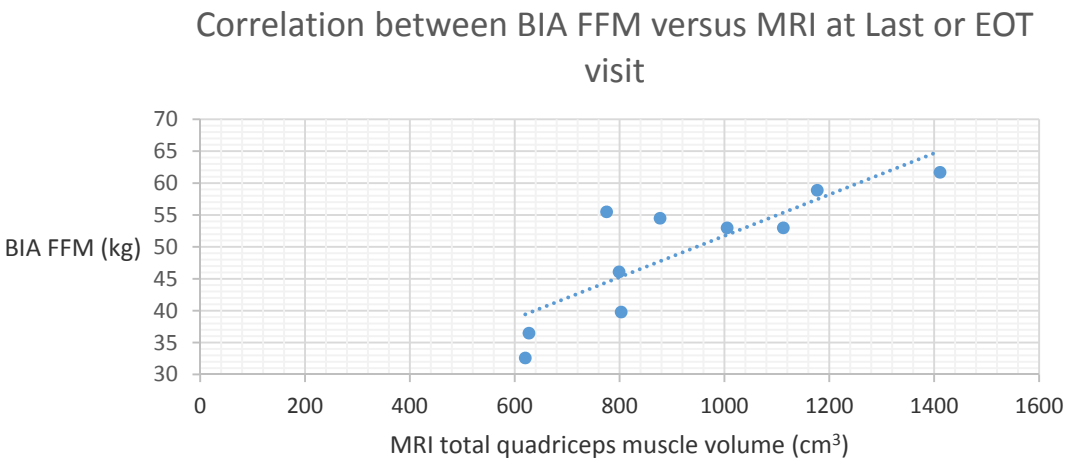
	Observer 1
Average slices per scan	98
Average time of analysis per scan (minutes)	61

Fig3 Graph of correlation of BIA FFM versus 3T MRI total quadriceps muscle volume at baseline



Six participants within Arm A and eleven within Arm B. Correlation=0.845.

Fig4 Graph of correlation of BIA FFM versus 3T MRI total quadriceps muscle volume at Last or EOT visit



Two participants within Arm A and eight within Arm B. Correlation=0.828.

Table 6 Intra-observer results for 3T MRI analysis for the ACCeRT study

Intra-observer (cm^3)				
	Observer 1 First analysis	**Observer 1 Second analysis**	**Difference**	**% difference**
Scan 02	1112	1123	+11	+0.99%
Scan 03	1011	987	-24	-2.37%
Scan 05	766	765	-1	-0.13%
Mean			**-4.67**	**-0.51%**

Difference and percentage difference between observer 1 second analysis and observer 1 first analysis, separated by one month

Table 7 Inter-observer results for 3T MRI analysis for the ACCeRT study

Inter-observer (cm^3)				
	Observer 1	**Observer 2**	**Difference**	**% difference**
Scan 01	1042	1038	-4	-0.38%
Scan 04	1252	1247	-5	-0.40%
Scan 05	766	766	0	0%
Mean			**-3**	**-0.26%**

Difference and percentage difference between observer 2 analysis and observer 1 analysis

Limitations

Limitations include both technical and human limitations. First; some participants have implanted metals, metallic stents, or prosthesis that are not MR safe (as per one participant with a cochlear implant allocated to Arm B), and second some patients are claustrophobic (34). Third, MRI scanning and analysis are dependent on the observer who performs the combined computerised and manual segmentation of each muscle within each slice. In the ACCeRT study, both observers agreed the start and end slice number on each scan, then independently performed the analysis. All scans were coded and blinded to treatment allocation. There was intra-observer agreement for three scans (10% of the total 30 scans, analysis separated by one month) with a mean difference of -4.67 cm^3 (-0.51%), and inter-observer difference of -3 cm^3 (-0.26%), all showing good correlation (Tables 6,7). Fourth; one participant scan was unable to be analyzed as per study protocol due to difficulty differentiating adipose tissue from muscle. Fifth: the two separate acquired volumes were combined from two separate acquisitions. This resulted in some mismatching when stitched together. This could have been changed to one complete acquisition which would have assisted in determining the fat saturation at the edge of the acquired volumes, along with reducing analysis time. The use of the 2-point Dixon-based protocol would also have assisted in the automated segmentation.

The results gained from this small, feasibility, single centre, open-label, randomised, NSCLC cancer cachexia study are only relevant in this population. The study attrition was high due to poor performance status and further progression of the participant's cancer. Utilising three MRI scan points within the ACCeRT study was discussed within the design stage, with scans at baseline, week 12 and week 20. Due to limited funding, this was reduced down to baseline and Last or EOT visit. This has resulted in the loss of potential data, along with whether the effect of net gain of the FFM seen by early BIA data was a true effect verified by MRI data. Coupled with the effect of attrition, especially within Arm A, no reasonable statement can be made.

Future studies could include more frequent scanning, either every 8 weeks or at 12 weeks, acknowledging that it is difficult to burden the patient especially in the end-stage/refractory cachexia population. Routine CT scanning can be used in pre-cachexia and cachexia populations, as usually the participants are still undergoing routine scanning as part of their anti-cancer treatment or surveillance concurrently with the cachectic treatment (3). Future studies investigating potential anabolic change could combine the L3-MRI i.e. abdomen and thigh muscle volume analysis within the same scan to reduce costs. Along with developing an acquisition protocol to assess both muscle quantity/volume and quality in terms of intramuscular adipose tissue.

Conclusions

ACCeRT is the first study to utilise 3T MRI total quadriceps muscle volume within a cancer cachexia study, along with the first in an end-stage/refractory cachexia population. These data suggests that there is a difference in muscle gained by gender and future studies should stratify for gender if muscle volume is the primary endpoint. These results can be used for baseline/reference for future cancer cachexia studies targeting the anabolic muscle pathways in end-stage/refractory cachexia patients.

Conflicts of interest

The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle - Clinical Reports (von Haehling S, Ebner N, Morley JE, Coats AJS, Anker SD. Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle - Clinical Reports. J Cachexia Sarcopenia Muscle Clinical Reports 2016; 1;e28:1-2.). This study has been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed consent prior to their inclusion in the study.

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Registration

Australian New Zealand Clinical Trials Registry; ACTRN12611000870954

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Competing interests

All authors declare that they have no competing interests.

Trial organisation

Auckland's Cancer Cachexia evaluating Resistance Training (ACCeRT) was designed and coordinated by the Department of General Practice and Primary Health Care, University of Auckland, New Zealand. University of Auckland is responsible for overall trial management, regulatory affairs, statistical planning and analysis, trial registration and reporting as well as quality assurance. Study sites include North Shore Hospice, Totara South Auckland Hospice, Clinical Research Centre, Grafton Campus, University of Auckland, New Zealand.

Medication supply

EPA was supplied by Metagenics (Aust) Pty Ltd, celecoxib (Celebrex®) by Pfizer Australia and New Zealand, and essential amino acids prepared by Musashi, Notting Hill, Australia.

Authors' contribution

ESR conceived the study and performed analysis as observer 2. ESR and RDM participated in the design of the main research study. WO performed the analysis as observer 1. AD and BP and RH developed the acquisition and analysis protocol. All authors read and approved the final manuscript.

List of abbreviations

ACCeRT	Auckland's Cancer Cachexia evaluating Resistance Training
BIA	Bioelectrical Impedance Analyser
CAMRI	Centre for Advanced Magnetic Resonance Imaging
COX-2	Cyclo-oxygenase-2
CSA	Cross sectional area
CT	Computed Tomography
DEXA	Dual-Energy X-ray Absorptiometry
EAAs	Essential amino acids
EPA	Eicosapentaenoic acid
EOT	End of Trial
ET	Endurance Training
FFM	Fat-Free Mass
FM	Fat Mass
LBM	Lean Body Mass
MRI	Magnetic Resonance Imaging
NSCLC	Non-Small Cell Lung Cancer
PRT	Progressive Resistance Training
ST	Strength Training

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