Osteoporosis and Sarcopenia 6 (2020) 139–145

Contents lists available at ScienceDirect

Osteoporosis and Sarcopenia

journal homepage: http://www.elsevier.com/locate/afos

Original article

Sarcopenia in women with hip fracture: A comparison of hormonal biomarkers and their relationship to skeletal muscle mass and function

Ming Li Yee [a, b, *], Raphael Hau [a, c], Alison Taylor [c], Mark Guerra [d], Peter Guerra [d], Peteris Darzins [a], Christopher Gilfillan [a, b]

[a] Eastern Health Clinical School, Monash University, Victoria, Australia
[b] Department of Endocrinology, Eastern Health, Victoria, Australia
[c] Department of Orthopaedic Surgery, Eastern Health, Victoria, Australia
[d] Department of Physiotherapy, Eastern Health, Victoria, Australia

ARTICLE INFO

Article history:
Received 2 March 2020
Received in revised form
13 June 2020
Accepted 21 June 2020
Available online 18 July 2020

Keywords:
Sarcopenia
Skeletal muscle
Hip fracture
Biomarker

ABSTRACT

Objectives: Sarcopenia is a decline in skeletal muscle mass and function. It is associated with adverse outcomes and increased mortality. Sarcopenia is also reported to be prevalent in the hip fracture population. Our aims in this study are to compare the hormonal profile in women with hip fracture to controls, and to assess the relationship between hormonal biomarkers to skeletal muscle mass and function in these women.

Methods: A cross sectional study was performed enrolling women above age 60 years old with hip fracture as a study group. For comparison healthy women from the community were recruited. Peripheral blood samples were obtained for analysis of hormonal profiles. Measures of skeletal muscle mass and function by muscle area on computed tomography, dual energy X-ray absorptiometry, bioelectrical impedance analysis, and grip strength was performed.

Results: A high proportion of sarcopenic individuals were detected in the hip fracture group (60%). Women with hip fracture compared to controls were older ($P = 0.073$), had lower serum albumin levels ($P < 0.001$), serum insulin-like growth factor-1 (IGF-1) ($P < 0.001$), insulin-like growth factor binding protein −3 (IGFBP-3) ($P < 0.001$), free testosterone levels ($P = 0.001$), and impaired beta cell function by homeostasis model assessment (HOMA beta) ($P = 0.038$).

Conclusions: There is a high proportion of sarcopenic individuals in the hip fracture group. Lowered serum levels of IGF-1 and IGFBP-3, HOMA beta cell function, and free testosterone levels were detected in this group and may serve as potential biomarkers of sarcopenia.

1. Introduction

Sarcopenia is a decline in skeletal muscle mass and function. It is more common in the older population but can also be seen in younger individuals with chronic diseases [1–3]. Since its initial description by Rosenberg in 1997 [4], sarcopenia is now listed as a condition in the International Classification of Diseases-10 [5], enabling better recognition and detection. Further research have also shown the association between sarcopenia and the risk of several adverse outcomes. These include prolonged hospital stay [6], increased falls and fracture risk [7,8], in addition to increased morbidity [9] and mortality [10–12]. Despite these findings, there are currently no pharmacological therapies for sarcopenia and this lack of progress likely relates to the complex balance between anabolic and catabolic pathways involved in skeletal muscle regulation [13].

Muscle growth is also affected by hormonal factors such as serum insulin like growth factor-1 (IGF-1) and testosterone [14]. A decline in these hormonal levels with increasing age contributes further to the loss of skeletal muscle mass with aging. In studies

* Corresponding author. Eastern Health Clinical School, Monash University, Level 2, 5 Arnold Street Box Hill and Department of Endocrinology, Eastern Health, 8 Arnold Street Box Hill, Victoria, 3128, Australia.
E-mail address: mingli.yee@monash.edu (M.L. Yee).
Peer review under responsibility of The Korean Society of Osteoporosis.

https://doi.org/10.1016/j.afos.2020.06.001

assessing growth hormone replacement in the older population, improvement in lean body mass but not muscle strength was observed [15], whilst physiological testosterone therapy in hypogonadal men only showed modest improvement in muscle mass and strength [16].

In older women, higher free testosterone levels were also associated with increased lean body mass [17]. Studies evaluating the use of dehydroepiandrosterone (DHEA), a precursor androgen, and its effects on muscle strength in older women have shown mixed results with 1 study reporting improvements in sitting leg strength and physical function assessed by the Short Physical Performance Battery Score [18], while another study did not detect any changes in muscle strength [19].

Hip fracture commonly affects older individuals with increased mortality risk in the subsequent year [20]. In those who survive, reduced quality of life and function have been reported [21–24]. The association between falls and fracture risk with sarcopenia is further strengthened through a recent systematic review [8], and the 5-year mortality risk is increased in those with concurrent sarcopenia and osteoporotic hip fractures [25]. Given the increased prevalence of sarcopenia in the hip fracture population [26–28], we performed a cross sectional study on this group, evaluating the hormonal profiles in these women and its association with skeletal muscle mass and strength.

We also assessed the relationships between hormonal levels to sarcopenia status (sarcopenia/no sarcopenia) by the European Working Group on Sarcopenia in Older People (EWGSOP) 1 criteria [29]. Since commencement of this project the EWGSOP criteria have been updated to EWGSOP2 [30]. Analysis was reported using both criteria.

The aim of this study is to describe and compare a panel of hormonal biomarkers related to skeletal muscle regulation in women with hip fractures, compared to controls. We hypothesized women with hip fractures compared to controls were more likely to be sarcopenic and have altered hormonal biomarker profiles related to skeletal muscle regulation.

2. Methods

Thirty-nine women aged above 60 years old were enrolled into the study between June 2016 and July 2019. Twenty women with hip fractures were recruited during their hospital admission as the study group. For comparison 19 healthy women from the community were recruited by community advertisements. All women also consented to collection of muscle biopsy for analysis of the molecular pathways involved in muscle regulation. These findings will be submitted as a separate paper. All participants provided informed consent prior to inclusion in the study. All procedures performed involving human participants were in accordance with the ethical standards of the Eastern Health Ethics Review Committee and was performed in accordance with the Helsinki Declaration of 1975, Eastern Health HREC reference number HREC/16/EH/104.

We were also interested in collecting data on length of stay on the rehabilitation ward (not reported in this study). As nursing home residents were generally discharged back to residential aged care facilities without a period of rehabilitation thus precluding the ability to collect this data, they were excluded from the study. Women with pre-existing neurological conditions which are known to affect skeletal muscle were also excluded. Women in the healthy control group were excluded if they were on blood thinning agents which would preclude muscle biopsy.

Sample size calculation was performed in reference to a study by Malkov [31] whereby there was 10% significant difference in muscle area reported in between groups. To detect a 10% difference in skeletal muscle cross sectional area between our groups, with a calculated power of 80% and 5% alpha, we estimated that a minimum of 31 women were required for the study. A total of 90 women were screened. Following exclusion, a total of 39 women were enrolled into the study.

Fasting peripheral blood samples were obtained for analysis of biochemistry, fasting glucose, insulin and hormonal levels related to skeletal muscle regulation. In women with hip fractures, fasting peripheral blood sample was collected within 1–5 days post-surgery. Frailty status were recorded using the Clinical Frailty Scale (CFS) [32,33]. This scale ranges from 1 (Very Fit) to 9 (Terminally Ill).

Skeletal muscle mass was assessed using dual energy X-ray absorptiometry (DXA) (Hologic Discovery, New South Wales, Australia), muscle cross sectional area on mid-thigh computed tomography (CT) scan (Aquillon Prime, Toshiba, Japan), and calculation of skeletal muscle index using the Janssen regression equation [34] utilising resistance data from bioelectrical impedance analysis machine (MDD1500, Body Stat Limited, Isle of Man).

Coefficient of variation for the DXA scan as provided by manufacturers are 1.2% lumbar spine, 1.4% total femur, and 1.9% femoral neck. Due to limitations around access to DXA imaging, all DXA was performed on an outpatient basis. DXA data were unavailable in 8 women from the hip fracture group who were either unable to tolerate the procedure or have declined to return for their imaging. In view of the relative lack of DXA data, prevalence of sarcopenia was assessed using data from bioelectrical impedance analysis (BIA).

Mid-thigh CT in the hip fracture group were performed within 1–3 days post-surgery. As there was a significant amount of muscle edema observed on the operated leg, muscle area from the non-operated leg was used for analysis in this study. In healthy controls an average of muscle area in both legs combined were used as a measure of skeletal muscle mass.

Skeletal muscle function by grip strength was measured on a Jamar handheld dynamometer. An average reading of 3 attempts were taken as the final grip strength.

The European Working Group on Sarcopenia in Older People (EWGSOP) 1 and 2 [29,30,34] criteria were used to define sarcopenia as all study subjects were Caucasian. Specific cut-off values used from both criteria to define sarcopenia are as listed:

EWGSOP1: Skeletal muscle mass index by BIA, adjusted for height <6.75 kg/m^2 and grip strength <20 kg.
EWGSOP 2: Skeletal muscle mass index by BIA, adjusted for height <6.75 kg/m^2 and grip strength <16 kg.

We have presented a parallel analysis using both criteria.

Student's T test were used to compare means between groups. Chi square test were used to compare sarcopenia status between groups. Analysis were performed using SPSS statistical software programme version 25 (IBM). A P value of < 0.05 was considered statistically significant.

The relationship between different biochemistry and hormonal variables to muscle mass and function were analyzed using univariate regression analysis followed by multiple regression analysis. As the groups differed by age, multiple regression analysis were performed adjusted for age as a confounder.

Binary logistic regression analysis were performed with the

participants further divided into sarcopenia status (yes/no) assessing the relationship between the different hormonal variables to sarcopenia status.

3. Results

3.1. Groupwise comparison: Participant's characteristics

Mean age in the whole group was 77.82 ± 10.31 years old (range 61–99). Women with hip fractures were older (80.70 ± 11.30 vs 74.79 ± 8.43 years, P = 0.073), frailer (3 ± 2 vs 2 ± 1, P < 0.001) and were on more medications (6 ± 4 vs 3 ± 2, P = 0.001) compared to healthy women from the community (Table 1).

Muscle cross sectional area (82.82 ± 16.61 vs 100.01 ± 19.09 cm^2, P = 0.005) and grip strength (14.01 ± 6.83 vs 22.93 ± 5.94 kg, P < 0.001) were lower in women in the hip fracture group, indicating increased sarcopenia risk in this group. A higher proportion of women in the hip fracture group were sarcopenic based on the EWSGOP1 criteria (60% vs 16% P = 0.005). Using EWGSOP2 criteria, 50% in the hip fracture group compared to 5% in the healthy controls were sarcopenic (P = 0.002) (Table 2).

3.2. Group wise comparison (hip fracture to healthy controls): biochemistry and hormonal profile

There was no difference in corrected calcium, parathyroid hormone, and vitamin D levels between groups. Albumin levels (30.25 ± 4.71 vs 40 ± 2.58 g/L, P < 0.001), free T3 (3.61 ± 0.73 vs 4.93 ± 0.74 pmol/L, P < 0.001), and free testosterone levels by mass spectrometry were significantly lower in the hip fracture group (5.00 ± 2.07 vs 8.85 ± 3.28 nmol/L, P-value = 0.001).

Women with hip fractures had comparatively lower serum IGF-1 (11.08 ± 4.11 vs 18.75 ± 5.48 nmol/L, P-value < 0.001), Growth Hormone (1.40 ± 1.75 vs 3.00 ± 3.31 ug/L, P = 0.065) and IGF Binding Protein 3 levels (IGFBP-3) (73.57 ± 28.08 vs 121.37 ± 28.60 nmol/L, P-value < 0.001) (Table 3a).

Elevated fasting glucose levels (6.04 ± 1.12 vs 5.43 ± 0.37 mmol/L, P = 0.035) and impaired beta cell function by homeostasis model assessment beta (HOMA Beta) (76.12 ± 21.72 vs 95.12 ± 29.77, P = 0.038) were detected in the hip fracture group (Table 3b). Three

Table 1
Demographics of participants.

Variable	Hip fracture (n = 20)	Healthy controls (n = 19)	P-value
Age, yr	80.70 ± 11.30	74.79 ± 8.43	0.073
Weight, kg	67.79 ± 13.32	67.57 ± 14.47	0.962
Body mass index, kg/m^2	26.27 ± 5.36	26.20 ± 4.56	0.968
Number of medication use	6 ± 4	3 ± 2	0.001
Clinical frailty scale score [a]	3 ± 2	2 ± 1	<0.001

Values are presented as mean ± standard deviation.
[a] Clinical frailty scale score: 1-Very fit, 2-Well, 3-Managing well, 4-Vulnerable, 5-Mildly frail, 6-Moderately frail, 7-Severely frail, 8-Very Severely frail, 9-Terminally ill.

Table 2
Skeletal muscle mass (muscle cross sectional area on CT and bioelectrical impedance analysis) and function (grip strength), and sarcopenia prevalence (by EWSGOP criteria).

Variable	Hip fracture (n = 20)	Healthy controls (n = 19)	P-value
Muscle cross sectional area: non operated leg or average reading of both legs combined, cm^2	82.82 ± 16.61	100.01 ± 19.09	0.005
Janssen skeletal muscle mass, kg	16.60 ± 3.49	17.70 ± 3.74	0.349
Dry lean mass by BIA, kg	5.78 ± 3.60	6.87 ± 2.52	0.279
Lean mass by DXA, kg	n = 12 36.85 ± 4.75	n = 19 38.18 ± 6.52	0.521
Grip strength, kg	14.01 ± 6.83	22.93 ± 5.94	<0.001
Sarcopenia by EWGSOP 1 criteria†	12 (60)	3 (16)	0.005
Sarcopenia by EWGSOP 2 criteria*	10 (50)	1([5)	0.002

Values are presented as mean ± standard deviation or number (%).
BIA, bioelectrical impedance analysis; DXA, dual energy X-ray absorptiometry; EWGSOP, European Working Group on Sarcopenia in Older People.
† Sarcopenia defined by EWGSOP 1 criteria: low skeletal muscle mass by BIA (skeletal muscle mass index < 6.75 kg/m^2) combined with low grip strength (<20 kg).
* Sarcopenia defined by EWGSOP 2 criteria: low skeletal muscle mass by BIA (skeletal muscle mass index < 6.75 kg/m^2) combined with low grip strength (<16 kg).

Table 3a
Comparison of biomarkers (biochemistry and hormonal levels) between groups.

Variable	Hip fracture (n = 20)	Healthy controls (n = 19)	P-value
Corrected calcium, mmol/L	2.43 ± 1.45	2.40 ± 0.63	0.388
Albumin, g/L	30.25 ± 4.71	40.00 ± 2.58	<0.001
Parathyroid Hormone, pmol/L	6.38 ± 3.69	6.56 ± 2.56	0.858
Vitamin D, nmol/L	58.70 ± 33.56	67 ± 23.10	0.373
Thyroid stimulating hormone, mU/L	2.35 ± 2.27	2.45 ± 2.08	0.890
Free T4, pmoL/L	18.13 ± 3.66	16.62 ± 2.78	0.157
Free T3, pmol/L	3.61 ± 0.73	4.93 ± 0.74	<0.001
Free testosterone, nmol/L	5.00 ± 2.07	8.85 ± 3.28	0.001
IGF-1, nmol/L	11.08 ± 4.11	18.75 ± 5.48	<0.001
Growth Hormone, ug/L	1.40 ± 1.75	3.00 ± 3.31	0.065
IGFBP-3, nmol/L	73.57 ± 28.08	121.37 ± 28.60	<0.001
IGF-1/IGFBP-3 ratio	0.17 ± 0.11	0.16 ± 0.03	0.657

Values are presented as mean ± standard deviation.
IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3.

women in this group were on therapy for diabetes; 2 on insulin and the other on a combination of metformin and a dipeptidyl peptidase 4 inhibitor. After exclusion individuals who were on insulin secretagogue and insulin treatment from the analysis, the reduced beta cell function observed in the hip fracture group was still maintained.

3.3. Regression analysis on the whole study population: hormonal variables to muscle mass and strength

Univariate regression analysis on the whole study population was performed to assess the relationship between variables which were significantly different between groups to skeletal muscle mass (muscle cross sectional area on CT) and muscle function (grip strength). To correct for the effect of age, regression analysis was also performed adjusting for age as a confounder. After correction for age, serum IGF-1, IGFBP-3 levels and HOMA beta were associated with skeletal muscle area on CT and grip strength (Table 4).

3.4. Comparison sarcopenic to non-sarcopenic women: biochemistry and hormonal profile

The whole population was divided into the sarcopenic and non-sarcopenic group by EWGSOP2 criteria for comparison of hormonal profile between sarcopenic to non-sarcopenic women. Albumin and serum IGFBP-3 levels were significantly lowered in the sarcopenic group ($P < 0.05$). There was a trend towards lower free testosterone levels, serum IGF-1 levels, and HOMA beta in the sarcopenic group. However, the difference between groups in these variables did not reach statistical significance ($P > 0.05$) (Table 5a). There was no significant difference detected in glucose or insulin levels when groups were divided by sarcopenia criteria by EWGSOP2 (Table 5b).

3.5. Binary logistic regression: hormonal variables to sarcopenia status

Binary logistic regression was performed on the whole population assessing the relationship between hormonal variables to sarcopenia status adjusted for age by EWGSOP1 criteria. For completion, analysis was also performed using the EWGSOP2 criteria. Results are reported as the odds of developing sarcopenia for every unit reduction in the independent variable.

When the population was assessed using the EWGSOP 1 criteria, insulin was the only variable which was significantly associated with sarcopenia status. For every 1mu/L decrease in insulin levels, the odds of developing sarcopenia increased by a factor of 1.40 (OR 1.40, P-value = 0.045) (Table 6). When the population was assessed using the EWGSOP2 criteria, age was a stronger predictor of sarcopenia.

4. Discussion

This study was performed on older women with hip fractures and were compared to healthy women from the community. Consistent with previous studies, there was a significant proportion of sarcopenic women in the hip fracture group. This was further supported by the presence of low muscle cross sectional area on mid-thigh CT and reduced grip strength. This confirms that women with hip fractures are a good model for sarcopenia in older women.

Low albumin levels, a marker of nutritional state were observed in the hip fracture group. These findings were consistent with other studies [35], and suggests impaired nutrition may be a risk factor for hip fracture. Alternatively, loss of albumin from inflammation and leakage into the extravascular space can occur in critically ill patients [36], and could be another reason for the low albumin levels seen in the hip fracture group.

Insulin-like growth factor-1 (IGF-1) positively affects skeletal muscle growth through its interaction and activation of the

Table 3b
Comparison of fasting glucose, insulin and beta cell function between groups.

Variable	Hip fracture (n = 17) [†]	Healthy controls (n = 19)	P-value
Fasting glucose, mmol/L	6.04 ± 1.14	5.43 ± 0.37	0.035
Fasting insulin, mU/L	9.58 ± 5.19	10.37 ± 5.86	0.663
HOMA Beta	76.12 ± 21.72	95.12 ± 29.77	0.038

Values are presented as mean ± standard deviation.
HOMA, Homeostasis Model Assessment.
[†] Individual of insulin secretagogue and insulin excluded from analysis.

Table 4
Univariate linear regression analysis with muscle area on CT and grip strength as dependent variables. [β] Analysis adjusted for age as a confounder.

Outcome variable	Independent variable	Unadjusted model		Adjusted model corrected for age [β]	
		Adjusted r^2	P-value (95% confidence interval)	Adjusted r^2	P-value (95% confidence interval)
Muscle area on CT, cm^2	Serum albumin	0.11	0.022 (0.17, 2.14)	0.11	0.059 (−0.03, 2.06)
	Serum IGF-1	0.17	0.007 (0.40, 2.33)	0.16	0.017 (0.24, 2.26)
	IGFBP-3	0.22	0.003 (0.09, 0.43)	0.21	0.013 (0.05, 0.43)
	HOMA Beta	0.31	<0.001 (0.21, 0.61)	0.34	<0.001 (0.21, 0.61)
	Free testosterone	0.19	0.006 (0.86,4.81)	0.19	0.018 (0.46, 4.58)
Grip Strength, kg	Serum albumin	0.34	<0.001 (0.41, 1.08)	0.61	0.001 (0.23, 0.77)
	Serum IGF-1	0.18	0.004 (0.20, 0.95)	0.55	0.012 (0.09, 0.66)
	IGFBP-3	0.28	0.001 (0.05, 0.19)	0.60	0.026 (0.01, 0.12)
	HOMA Beta	0.08	0.051 (0.00, 0.18)	0.56	0.008 (0.03, 0.15)
	Free testosterone	0.15	0.016 (0.21, 1.89)	0.58	0.078 (−0.06, 1.17)

CT, computed tomography; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; HOMA, Homeostasis Model Assessment.

Table 5a
Comparison of biomarkers (biochemistry and hormonal levels) between sarcopenic and non-sarcopenic groups.

Variable	Sarcopenic (n = 11)	Non Sarcopenic (n = 28)	P-value
Corrected calcium, mmol/L	2.43 ± 0.16	2.41 ± 0.09	0.666
Albumin, g/L	30.73 ± 6.17	36.68 ± 5.47	0.005
Parathyroid Hormone, pmol/L	7.44 ± 3.52	6.09 ± 2.97	0.234
Vitamin D, nmol/L	58.46 ± 38.00	64.43 ± 25.07	0.638
Thyroid stimulating hormone, mU/L	3.33 ± 2.68	2.03 ± 1.84	0.093
Free T4, pmol/L	18.74 ± 3.67	16.87 ± 3.05	0.113
Free T3, pmol/L	3.66 ± 0.86	4.49 ± 0.95	0.017
Free testosterone, nmol/L	5.60 ± 1.70	7.08 ± 3.67	0.234
IGF-1, nmol/L	12.09 ± 5.14	15.89 ± 6.26	0.082
Growth Hormone, ug/L	1.32 ± 1.58	2.52 ± 3.00	0.220
Insulin like Growth Factor Binding Protein 3, nmol/L	69.94 ± 28.27	104.24 ± 35.57	0.011
IGF-1/IGFBP3 ratio	0.17 ± 0.05	0.17 ± 0.10	0.879

Values are presented as mean ± standard deviation.
IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3.

Table 5b
Comparison of fasting glucose, insulin and beta cell function between sarcopenic and non-sarcopenic groups.

Variable	Sarcopenic (n = 11)	Non Sarcopenic † (n = 25)	P-value
Fasting glucose, mmol/L	5.87 ± 0.68	5.68 ± 0.97	0.549
Fasting insulin, mU/L	8.64 ± 3.44	10.52 ± 6.09	0.343
HOMA Beta	75.06 ± 22.93	91.02 ± 28.53	0.112

Values are presented as mean ± standard deviation.
† Individual on insulin secretagogue and insulin excluded from analysis.

Table 6
Binary logistic regression analysis with sarcopenia status by EWGSOP1 criteria, adjusted for age.

Outcome variable	Independent variable adjusted for age	OR	P-value (95% confidence interval)
Sarcopenia Status (EWGSOP1)	Serum albumin	1.16	0.061 (0.99, 1.34)
	Serum IGF-1	1.05	0.549 (0.90, 1.22)
	IGFBP-3	1.02	0.186 (0.99,1.06)
	HOMA Beta	1.01	0.377 (0.98, 1.05)
	Free testosterone	1.31	0.216 (0.86, 2.01)
	Serum insulin levels	1.40	0.045 (1.01, 1.93)

EWGSOP, European Working Group on Sarcopenia in Older People.
Sarcopenia defined by EWGSOP 1 criteria: low skeletal muscle mass by BIA (skeletal muscle mass index < 6.75 kg/m^2) combined with low grip strength (<20 kg).
IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; HOMA, Homeostasis Model Assessment.

mammalian target of rapamycin (mTOR) pathway. Unbound IGF-1 has a short half-life, and thus the majority of IGF-1 in the circulation is bound to IGF binding proteins [37]. IGF binding protein 3 (IGFBP-3), being the most abundant in the circulation, was measured in our study to provide further information. In our cohort, IGF-1 and IGFBP-3 were both positively related to skeletal muscle mass and function, suggesting a stimulatory effect on muscle growth. These findings combined with lowered IGF-1 and IGFBP-3 levels observed in the our hip fracture group were similar to findings from previous studies in hip fracture [38] and stroke patients [39]. Additionally, animal models have observed the protective effect of IGF-1 and IGFBP-3 on the muscle [40], whilst cell culture models suggests IGFBP-3 upregulates the mTOR pathway [41]. The positive relationship observed between IGF-1, IGFBP-3 to skeletal muscle mass and function may suggest a protective effect of raised IGF-1 and IGFBP-3 against sarcopenia, although this requires further study.

Free testosterone levels by mass spectrometry were positively associated with skeletal muscle mass and strength and were observed to be lowest in our hip fracture cohort. Using DXA and bioelectrical impedance analysis, the positive relationship between elevated free testosterone levels and lean body mass have been reported before [17,42]. Our analysis strengthens this finding further by showing the positive association between free testosterone levels to skeletal muscle mass on CT, a gold standard imaging tool for sarcopenia.

There was a positive relationship between free testosterone to grip strength. However, this relationship became non-significant after adjustment for age, suggesting that there were other factors contributing to the loss of muscle function with age. In this setting, free testosterone levels was a better indicator of low skeletal muscle mass but not reduced function.

Another finding in our study is that of lowered beta cell function by the homeostasis model, an indicator of intrinsic insulin production in the hip fracture group. Insulin is secreted by the pancreatic beta cells and works by enhancing glucose uptake into the target cells through its effect on the glucose transporter. Insulin appears to have several roles; glucose homeostasis and protein synthesis, and likely has an indirect effect on the bone. In muscle, in the presence of amino acids, insulin prevents protein breakdown and increases protein synthesis through activation of the mTOR 1 signalling [43–45]. In our study HOMA beta remained significantly associated with skeletal muscle area and function independent of age. This finding combined with the positive association between insulin to sarcopenia status further supports the role of insulin on the skeletal muscle.

When a comparison of hormonal levels was performed with the population divided into sarcopenic and non-sarcopenic individuals by EWGSOP2 criteria, IGFBP-3 and albumin levels were significantly lower in sarcopenic women. While low levels of albumin are

indicative and consistent with sarcopenia, low IGFBP-3 detected in sarcopenic women supports a likely link with the skeletal muscle as highlighted earlier in the discussion.

Analysis by binary logistic regression assessing the relationship between hormonal variables to sarcopenia status was performed using EWGSOP 1 and 2 criteria. When adjusted for age using EWGSOP1 criteria, insulin was associated with increased odds of sarcopenia. However, this relationship became non-significant when analysis was performed using EWGSOP2 criteria indicating age as a stronger predictor of sarcopenia status by EWGSOP2 criteria.

Another alternative explanation is that the EWGSOP2 criteria may underestimate sarcopenia in our population. Amendments to grip strength criteria in EWGSOP2 was made based on a study [46] which was performed in populations in the United Kingdom. To our knowledge at present there is no normative data for grip strength for the Australian population. Villani [47] recently compared both EWGSOP criteria in an Australian population. They report a significant discordance between both definitions of sarcopenia. Given this finding further studies are needed to further evaluate and compare both EWGSOP 1 and 2 criteria in the Australian population.

To summarise our study findings, women with hip fractures have a high prevalence of sarcopenia. To our knowledge, impaired beta cell function and low free testosterone levels have not been reported in the hip fracture cohort and serves as new knowledge. Whilst low IGF-1 and IGFBP-3 levels been reported in hip fracture patients before, the mechanisms underlying these findings requires further research.

Our study had several limitations. We were unable to age match our study participants, hence limiting direct comparison between groups. Despite this, our observations can still shed light on mechanisms of skeletal muscle loss with age. Blood samples for patients with hip fracture patients were collected between day 1–5 post-operatively, potentially confounding our results with the effects of acute surgical stress. We did not have follow up blood samples for hormonal analysis which would allow further comparison of hormone levels in the non-stressed state. Our lack of DXA data limits our ability to use this as a measure of sarcopenia in our cohort and therefore further analysis. It is possible that those who were able to return for their DXA scans post-operatively were less frail and less sarcopenic. This thus explains the lack of difference seen in skeletal muscle mass between groups by DXA data. Although dry lean mass measurement by bioelectrical electrical impedance analysis were available for all participants, we were unable to show a statistically significant difference between groups. This likely relates to our small sample size further limiting the generalisability of our study findings.

Further work would involve exploring the relationship between the IGF-1 pathway, insulin homeostasis and its effect on skeletal muscle regulation in these women.

5. Conclusions

Women with hip fractures have a high prevalence of sarcopenia. Low serum IGF-1, IGFBP-3, free testosterone and impaired beta cell function were detected in these women and may serve as potential biomarkers for sarcopenia. This is an area for future research.

Conflicts of interest

This study was funded by Monash University Victoria Australia and the Elaine and Frank Derwent Memorial Research Grant Eastern Health Foundation, Victoria Australia reference EHFRG2017_002. No other potential conflict of interest relevant to this article was reported.

CRediT author statement

Ming Li Yee: Formal analysis, Resources, Writing - Original Draft, Writing - Review & Editing. **Raphael Hau**: Resources. **Alison Taylor**: Resources. **Mark Guerra**: Resources. **Peter Guerra**: Resources. **Peteris Darzins**: Resources. **Christopher Gilfillan**: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Supervision.

Acknowledgments

We would like to acknowledge our study participants, the orthopaedic surgeons and registrars at the Eastern Health orthopaedic department for their involvement in the study. We thank Dr Jim Doery and Mr Michael Daskalakis at Monash Health for their assistance with analysis of serum samples for free testosterone levels. **ORCID** Ming Li Yee: 0000-0003-0742-5493. Raphael Hau: 0000-0002-7380-7812. Alison Taylor: 0000-0003-1617-7671. Mark Guerra: 0000-0002-9316-4033. Peter Guerra: 0000-0003-4404-7145. Peteris Darzins: 0000-0001-8554-7432. Christopher Gilfillan: 0000-0002-8193-8994.

References

[1] Ryan E, McNicholas D, Creavin B, Kelly ME, Walsh T, Beddy D. Sarcopenia and inflammatory bowel disease: a systematic review. Inflamm Bowel Dis 2019;25:67–73.
[2] Park YS, Kim JW, Kim BG, Lee KL, Lee JK, Kim JS, et al. Sarcopenia is associated with an increased risk of advanced colorectal neoplasia. Int J Colorectal Dis 2017;32:557–65.
[3] Toptas M, Yalcin M, Akkoc I, Demir E, Metin C, Savas Y, et al. The relation between sarcopenia and mortality in patients at intensive care unit. BioMed Res Int 2018;2018:5263208.
[4] Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr 1997;127(5 Suppl). 990S-1S.
[5] Anker SD, Morley JE, von Haehling S. Welcome to the ICD-10 code for sarcopenia. J Cachexia Sarcopenia Muscle 2016;7:512–4.
[6] Gariballa S, Alessa A. Sarcopenia: prevalence and prognostic significance in hospitalized patients. Clin Nutr 2013;32:772–6.
[7] Clynes MA, Edwards MH, Buehring B, Dennison EM, Binkley N, Cooper C. Definitions of sarcopenia: associations with previous falls and fracture in a population sample. Calcif Tissue Int 2015;97:445–52.
[8] Yeung SSY, Reijnierse EM, Pham VK, Trappenburg MC, Lim WK, Meskers CGM, et al. Sarcopenia and its association with falls and fractures in older adults: a systematic review and meta-analysis. J Cachexia Sarcopenia Muscle 2019;10:485–500.
[9] Jones K, Gordon-Weeks A, Coleman C, Silva M. Radiologically determined sarcopenia predicts morbidity and mortality following abdominal surgery: a systematic review and meta-analysis. World J Surg 2017;41:2266–79.
[10] Englesbe MJ, Patel SP, He K, Lynch RJ, Schaubel DE, Harbaugh C, et al. Sarcopenia and mortality after liver transplantation. J Am Coll Surg 2010;211:271–8.
[11] Filippin LI, Teixeira VN, da Silva MP, Miraglia F, da Silva FS. Sarcopenia: a predictor of mortality and the need for early diagnosis and intervention. Aging Clin Exp Res 2015;27:249–54.
[12] Landi F, Cruz-Jentoft AJ, Liperoti R, Russo A, Giovannini S, Tosato M, et al. Sarcopenia and mortality risk in frail older persons aged 80 years and older: results from ilSIRENTE study. Age Ageing 2013;42:203–9.
[13] Sakuma K, Aoi W, Yamaguchi A. Molecular mechanism of sarcopenia and cachexia: recent research advances. Pflügers Archiv 2017;469:573–91.
[14] Morley JE. Hormones and sarcopenia. Curr Pharmaceut Des 2017;23:4484–92.
[15] De Spiegeleer A, Beckwee D, Bautmans I, Petrovic M. Sarcopenia guidelines development group of the Belgian society of G, geriatrics. Pharmacological interventions to improve muscle mass, muscle strength and physical performance in older people: an umbrella review of systematic reviews and meta-analyses. Drugs Aging 2018;35:719–34.
[16] Borst SE. Interventions for sarcopenia and muscle weakness in older people. Age Ageing 2004;33:548–55.
[17] Rariy CM, Ratcliffe SJ, Weinstein R, Bhasin S, Blackman MR, Cauley JA, et al. Higher serum free testosterone concentration in older women is associated with greater bone mineral density, lean body mass, and total fat mass: the cardiovascular health study. J Clin Endocrinol Metab 2011;96:989–96.
[18] Kenny AM, Boxer RS, Kleppinger A, Brindisi J, Feinn R, Burleson JA. Dehydroepiandrosterone combined with exercise improves muscle strength and

physical function in frail older women. J Am Geriatr Soc 2010;58:1707–14.
[19] Morales AJ, Haubrich RH, Hwang JY, Asakura H, Yen SS. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. Clin Endocrinol 1998;49:421–32.
[20] Katsoulis M, Benetou V, Karapetyan T, Feskanich D, Grodstein F, Pettersson-Kymmer U, et al. Excess mortality after hip fracture in elderly persons from Europe and the USA: the CHANCES project. J Intern Med 2017;281:300–10.
[21] Center JR. Fracture burden: what two and a half decades of dubbo osteoporosis epidemiology study data reveal about clinical outcomes of osteoporosis. Curr Osteoporos Rep 2017;15:88–95.
[22] Milte R, Crotty M, Miller MD, Whitehead C, Ratcliffe J. Quality of life in older adults following a hip fracture: an empirical comparison of the ICECAP-O and the EQ-5D-3 L instruments. Health Qual Life Outcome 2018;16:173.
[23] Guirant L, Carlos F, Curiel D, Kanis JA, Borgstrom F, Svedbom A, et al. Health-related quality of life during the first year after a hip fracture: results of the Mexican arm of the international cost and utility related to osteoporotic fractures study (MexICUROS). Osteoporos Int 2018;29:1147–54.
[24] Alexiou KI, Roushias A, Varitimidis SE, Malizos KN. Quality of life and psychological consequences in elderly patients after a hip fracture: a review. Clin Interv Aging 2018;13:143–50.
[25] Kim YK, Yi SR, Lee YH, Kwon J, Jang SI, Park SH. Effect of sarcopenia on postoperative mortality in osteoporotic hip fracture patients. J Bone Metab 2018;25:227–33.
[26] Steihaug OM, Gjesdal CG, Bogen B, Kristoffersen MH, Lien G, Ranhoff AH. Sarcopenia in patients with hip fracture: a multicenter cross-sectional study. PloS One 2017;12:e0184780.
[27] Yoo JI, Ha YC, Kwon HB, Lee YK, Koo KH, Yoo MJ. High prevalence of sarcopenia in Korean patients after hip fracture: a case-control study. J Kor Med Sci 2016;31:1479–84.
[28] Gonzalez-Montalvo JI, Alarcon T, Gotor P, Queipo R, Velasco R, Hoyos R, et al. Prevalence of sarcopenia in acute hip fracture patients and its influence on short-term clinical outcome. Geriatr Gerontol Int 2016;16:1021–7.
[29] Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. Age Ageing 2010;39:412–23.
[30] Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2019;48:16–31.
[31] Malkov S, Cawthon PM, Peters KW, Cauley JA, Murphy RA, Visser M, et al. Hip fractures risk in older men and women associated with DXA-derived measures of thigh subcutaneous fat thickness, cross-sectional muscle area, and muscle density. J Bone Miner Res 2015;30:1414–21.
[32] Rockwood K, Song X, MacKnight C, Bergman H, Hogan DB, McDowell I, et al. A global clinical measure of fitness and frailty in elderly people. CMAJ (Can Med Assoc J) 2005;173:489–95.
[33] Juma S, Taabazuing MM, Montero-Odasso M. Clinical frailty scale in an acute medicine unit: a simple tool that predicts length of stay. Can Geriatr J 2016;19:34–9.
[34] Janssen I, Heymsfield SB, Baumgartner RN, Ross R. Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol 1985;89:465–71. 2000.
[35] Diaz de Bustamante M, Alarcon T, Menendez-Colino R, Ramirez-Martin R, Otero A, Gonzalez-Montalvo JI. Prevalence of malnutrition in a cohort of 509 patients with acute hip fracture: the importance of a comprehensive assessment. Eur J Clin Nutr 2018;72:77–81.
[36] Caironi P, Gattinoni L. The clinical use of albumin: the point of view of a specialist in intensive care. Blood Transfus 2009;7:259–67.
[37] Bach LA. Insulin-like growth factor binding proteins-an update. Pediatr Endocrinol Rev 2015;13:521–30.
[38] Boonen S, Mohan S, Dequeker J, Aerssens J, Vanderschueren D, Verbeke G, et al. Down-regulation of the serum stimulatory components of the insulin-like growth factor (IGF) system (IGF-I, IGF-II, IGF binding protein [BP]-3, and IGFBP-5) in age-related (type II) femoral neck osteoporosis. J Bone Miner Res 1999;14:2150–8.
[39] Silva-Couto Mde A, Prado-Medeiros CL, Oliveira AB, Alcantara CC, Guimaraes AT, Salvini Tde F, et al. Muscle atrophy, voluntary activation disturbances, and low serum concentrations of IGF-1 and IGFBP-3 are associated with weakness in people with chronic stroke. Phys Ther 2014;94:957–67.
[40] Lang CH, Frost RA, Svanberg E, Vary TC. IGF-I/IGFBP-3 ameliorates alterations in protein synthesis, eIF4E availability, and myostatin in alcohol-fed rats. Am J Physiol Endocrinol Metab 2004;286:E916–26.
[41] Hong S, Kim MM. IGFBP-3 plays an important role in senescence as an aging marker. Environ Toxicol Pharmacol 2018;59:138–45.
[42] Yakabe M, Kojima T, Okumura T, Takiyama S, Umeda-Kameyama Y, Akishita M, et al. Serum free testosterone levels are positively correlated with skeletal muscle mass in older women aged over 75 years. Geriatr Gerontol Int 2019;19:460–1.
[43] Timmerman KL, Lee JL, Dreyer HC, Dhanani S, Glynn EL, Fry CS, et al. Insulin stimulates human skeletal muscle protein synthesis via an indirect mechanism involving endothelial-dependent vasodilation and mammalian target of rapamycin complex 1 signaling. J Clin Endocrinol Metab 2010;95:3848–57.
[44] Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. Am J Physiol Endocrinol Metab 2008;295:E595–604.
[45] Everman S, Meyer C, Tran L, Hoffman N, Carroll CC, Dedmon WL, et al. Insulin does not stimulate muscle protein synthesis during increased plasma branched-chain amino acids alone but still decreases whole body proteolysis in humans. Am J Physiol Endocrinol Metab 2016;311:E671–7.
[46] Dodds RM, Syddall HE, Cooper R, Benzeval M, Deary IJ, Dennison EM, et al. Grip strength across the life course: normative data from twelve British studies. PloS One 2014;9:e113637.
[47] Villani A, McClure R, Barrett M, Scott D. Diagnostic differences and agreement between the original and revised European Working Group (EWGSOP) consensus definition for sarcopenia in community-dwelling older adults with type 2 diabetes mellitus. Arch Gerontol Geriatr 2020;89:104081.