Original Article

Circulating C-Terminal Agrin Fragment: A Potential Marker for Sarcopenia Among Type 2 Diabetes

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Abstract

Background: Undetected onset of sarcopenia among individuals with chronic diseases especially Type 2 Diabetes Mellitus (T2D) makes it important to be evaluated. The feasibility of diagnosing sarcopenia in a clinical setup might be a difficult task. Circulating markers including C-terminal agrin fragment (CAF) are emerging as an alternative. Hence, the objectives of the study were to compare circulating CAF levels between T2D, prediabetes (PD) and healthy controls and to study its association with sarcopenic index, muscle mass, strength and quality. **Methods:** Ninety-nine participants (n = 42, T2D; n = 33, PD; n = 24, healthy controls) aged 18 to 60 yrs were recruited. HOMA (homeostatic model assessment) indices were derived using plasma glucose and insulin. All participants underwent lipid profiling, muscle strength including quality (isokinetic dynamometer), body composition (Dual energy X-ray Absorptiometry (DXA)) and sarcopenic index (appendicular skeletal muscle mass/body weight) assessment. Serum samples were used to estimate CAF levelsusing enzyme-linked immunosorbent assay (ELISA). **Results:** Median CAF level was significantly higher among T2D group compared to PD and control groups ($P < 0.0001$). Circulating CAF levels correlated positively with age and glycated haemoglobin (HbA1c) (both, $P < 0.001$) and negatively with HOMA-B and muscle quality (both, $P < 0.001$), and sarcopenic index ($P = 0.07$). Multivariable analysis demonstrated that the odds of being in the highest tertile category was 7.67, 95% C.I. (2.10, 29.3) among T2D. **Conclusion:** Circulating CAF levels were significantly higher among T2D compared to PD and control study groups along with reduced skeletal muscle quality. This suggests that the circulating CAF level has the potential to be considered as a clinical marker to evaluate sarcopenia among T2D.

Keywords: C terminal agrin, diabetes, sarcopenia, skeletal muscle

Introduction

The loss of skeletal muscle functionality and its poorer metabolic health with altered glucose disposal has been noted in Type 2 Diabetes (T2D).[1] Supporting the same, our data showed that the maximum muscle strength and contractile quality using an isokinetic dynamometer were significantly lower in T2D and prediabetes (PD) compared to healthy controls, though all the three groups had comparable lower limb muscle mass. It was shown that the contractile quality was significantly and inversely associated with insulin resistance.[2] Poorer skeletal muscle functionality irrespective of normal muscle mass was intriguing which drove us to study the changes of skeletal muscle using invasive biopsy techniques. We published the first direct evidence of the existence of significantly higher intramyocellular lipid levels within skeletal muscle cells among normal and overweight young Indians with PD.[3] The onset of changes in skeletal muscle at PD stage itself reflected the poor quality of skeletal muscle.

This is in accordance with other studies which state that T2D-associated sarcopenia and frailty-related changes are projected to be almost 3 to 16 times higher compared to healthy aging.[4] Sarcopenia is an age-related decline in skeletal muscle mass and strength,[5] the onset of which could be much earlier especially in T2D, maybe at PD stage itself. Hence, its timely

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assessment routinely as a part of clinical practice is warranted. Gold standard methods such as an isokinetic dynamometer to assess skeletal muscle strength could be a time-consuming and invasive technique like muscle biopsy to study the molecular changes is not feasible. These techniques come at a cost with a lower proportion of the population being diagnosed and treated. The major concern of lack of evaluation is the adverse health consequences related to sarcopenia.[6] Therefore, the need for an accessible cost-effective biochemical marker that can be measured in bio-fluids to identify and monitor sarcopenia is necessary. This could potentially be an essential step forward in the management of sarcopenia.[7]

The proposed circulating C-terminal agrin fragment (CAF) estimation has been shown to be elevated in conditions associated with sarcopenia.[8-11] Disruption of neuro muscular junction (NMJ) integrity via proteolytic cleavage of Agrin into CAF22 detected easily in circulation has been noted in sarcopenia and other catabolic conditions and hence was chosen.[8] Serum CAF has never been evaluated among Indians, especially with T2D. This will be the first study to evaluate serum CAF levels in a cohort of PD and T2D, and compare them with healthy controls. There is a lack of studies measuring muscle mass and muscle strength using methods including the Dual energy X-ray Absorptiometry (DXA) and isokinetic dynamometry among the cohort of PD and T2D and associating it with circulating CAF levels among Indians. This circulating marker could be of relevance to clinicians, as it has the potential to identify early changes of sarcopenia. The emphasis on aging with chronic diseases including T2D must shift towards a sustainable ecosystem for a T2D patient to lead an independent and healthy life. This could reduce the burden not only at an individual level but also at the community level.

Therefore, the objectives of the present study are (i) to compare circulating CAF levels among T2D, PD and healthy controls and (ii) to study the strength of association of sarcopenic index, muscle mass, strength, quality and glycemic status with circulating CAF levels.

Methods

Ninety-nine subjects (42, T2D; 33, PD; 24 healthy controls) between the ages of 18 to 60 years with a BMI of 18.5 to 30 kg/m^2 were recruited from in and around the Medical College and Hospital. The American Diabetes Association Expert Committee criteria were used to diagnose PD and T2D.[12] Participants with any other chronic disease (i.e. hypertension, tuberculosis, cancer, chronic renal failure and ischemic heart disease), anaemia, nephropathy, peripheral neuropathy, muscular dystrophies, joint injuries and weight loss greater than 2 kg in the past 6 months were excluded from the study. The medication history of T2D participants was collected and the details are as follows: n=12, were taking Tab Metformin; n=7, were taking sulfonylureas; n=1 was on DPP-4 inhibitor; n=1 was on SGLT-2 inhibitor; n=12 were not taking drugs; n=18, no history was available. All protocols were approved by the Institutional Review Board and written informed consent was obtained from all participants before the study maintaining their anonymity and confidentiality.

The plasma sample was used for the estimation of glucose [GOD-POD method (Beckman Coulter AU480, Japan)] and insulin [electrochemiluminescence (Elecsys 2010, Roche Diagnostics, Manheim, Germany)].[13] Insulin resistance and beta-cell function [HOMA-IR (homeostatic model assessment-insulin resistance) and HOMA-% B] were assessed by the homeostatic method using standard formulae for calculation.[14] Serum lipid profiling [serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride] was measured by the Chemiluminescence Immunoassay (Siemens, Model EXL with LM 1 and 2, Germany).

Weight (Salter digital scale, 9069 PK3R, Tonbridge, UK) and height (Holtain stadiometer, Crymych, UK) of participants were recorded. Body composition (lean soft tissue mass, fat mass in kg and bone mineral density for both the whole body and specific regions) was assessed using DXA (Model DPXMD 7254, Lunar Corporation, Madison, WI). Appendicular muscle mass (AMM) was equivalent to the sum of lean soft tissue in both the right and left arms and legs was also measured.[15] Sarcopenia index was derived using the following equation skeletal muscle AMM/weight, skeletal muscle AMM/height^2 (kg/m^2).[16]

Isokinetic dynamometry (Kin Com AP1, Chattanooga Group, Tennessee, USA) was used to assess isometric and isokinetic knee extensor strength. The best of the three maximal voluntary isometric contractions for knee extension with the knee extended 30 degrees from the 90 degrees flexed position and dynamic (isokinetic) peak torque (60 degree/s) were used for analysis. Peak torque data were normalized to AMM (DXA scan) of the respective limb to derive muscle quality (Nm/kg).[17] Muscle-to-fat ratio is calculated by using the formula lean mass (kg) by body fat (kg) which is estimated by DXA.

The serum CAF levels were estimated by the 96-well plate ELISA method (FineTest, Wuhan Fine Biotech Co., Ltd. China, Cat. no. EH4820). The 96-well plate is pre-coated with Anti-CAF antibody and the biotin conjugated anti-CAF antibody was used as a detection antibody. The standards, test samples (~100 ul of serum) and controls were directly added to the respective wells, sealed with sealing films and incubated at 37°C (Heratherm Incubator, ThermoScientific, Germany) for 90 min. The plate was washed with wash buffer after discarding the plate contents followed by the addition of a biotin-conjugated detection antibody that was again incubated at 37°C for 60 min. After the wash procedure, the HRP-Streptavidin solution was added to each well and incubated for 30 min followed by a final washing of the plate to remove the unbound conjugates. TMB substrate was added to each well, sealed and incubated in dark at 37°C for 15 min to develop the blue colour that was directly proportional to the CAF amount of sample captured in the plate. The

reaction was further stopped using the acidic stop solution that changed the blue colour into yellow. The O.D. absorbance was measured spectrophotometrically at 450 nm in a microplate reader (Synergy H1 microplate reader, BioTek, USA) and then the concentration of CAF in the samples was calculated using the calibration graph generated independently on each plate.[18] The Intra-assay and Inter-assay CV (coefficient of variation) for the test was <5.6% and <6.9%, respectively. The samples were analysed in duplicates, that is, testing of samples for CAF two times by ELISA and mean values were reported, with the CV of duplicates <5%. The CAF assay ranges between 23.44 and 3000 pg/ml with the lowest detectable level (distinguishable from zero with 95% confidence) as 9 pg/ml.

Sample size: Sample size estimation was based on the closest literature available that compared the CAF levels between sarcopenic and control subjects, Al Rubaye *et al.*, 2020.[9] The mean difference in CAF levels between sarcopenia individuals and healthy control was found to be 5 pg/ml units. Expecting a mean difference of 5 pg/ml units in CAF (pg/ml) levels with SD of 6.1 and 3.9 pg/ml between the sarcopenia individuals and healthy control respectively, after accounting for multiple comparisons, with a 5% level of significance and 80% power, the sample size required was 22 in each group.

Statistical methods

Descriptive statistics were reported as mean with SD for the normally distributed continuous data and median with 25th and 75th percentiles for variables that were not normally distributed. Analysis of variance was performed to compare the physiological, biochemical and muscle strength variables between the study groups. Post-hoc multiple comparisons were performed using Bonferroni correction. Kruskal–Wallis test was used to compare the circulating CAF levels between the study groups. A Chi-square test was used to test the association between the tertiles of CAF with the study groups. Multivariable ordinal regression was performed to assess the factors associated with the highest tertiles of CAF. A *P* value less than 5% was considered statistically significant. All the analyses were carried out using SPSS version 25.0.

The instituitional ethics committee has approved the study. Date of approval is 7th September 2017.

Results

Table 1 describes the comparison of physiological, and biochemical and muscle strength variables between T2D, PD and control groups. The mean age of the participants was significantly different between the study groups ($P < 0.001$). Mean body weight, BMI, body fat and muscle-to-fat ratio were comparable among the three study groups. Lean mass and AMM were significantly lower among T2D compared to the PD and control group ($P < 0.01$). Serum cholesterol, HDL and LDL were comparable among the three study groups except serum triglycerides and were significantly higher among the T2D study group ($P = 0.02$). HbA1c, fasting glucose, HOMA B and HOMA-IR were significantly higher in T2D as compared to the PD and control groups ($P < 0.001$). Muscle strength including isometric and isokinetic was significantly lower in T2D as compared to the control group ($P < 0.001$).

The circulating CAF negatively correlated with lean body mass, fat mass and gynoid fat; positively correlated with android fat and bone mineral density which was not statistically significant. Median CAF levels were significantly higher among the T2D study group as compared to the PD and control group ($P < 0.0001$), with no significant difference between the PD and control groups. CAF was positively and significantly correlated with age ($r = 0.46$, $P < 0.001$) and HbA1c ($r = 0.54$, $P < 0.001$) and negatively correlated with HOMA-B ($r = -0.43$, $P < 0.001$), muscle quality isometric 60 degree/s ($r = -0.21$, $P < 0.001$) and isokinetic ($r = -0.22$ $P < 0.001$) measures, and sarcopenic index ($r = -0.15$, $P = 0.07$). Considering the skewness of the CAF data, CAF was categorized into tertiles and its association with the study group is presented in Figure 1. A significant association was noted between the three study groups with the tertiles of CAF ($P < 0.0001$). It was noted that participants belonging to the highest tertiles of CAF were significantly over-presented in the T2D group, compared to participants in the other two lowest categories of tertiles who were proportionately higher in PD and control groups ($P < 0.0001$). Table 2 represents the results of ordinal regression analysis, including the factors associated with the highest CAF tertile. Ordinal regression analysis adjusted for age, gender and BMI, the odds of being in CAF highest tertiles was 7.67 and 95% C.I. (2.10, 29.3) higher among subjects being T2D compared to other groups. Higher age was more likely to be significantly associated with the highest tertile of CAF (AOR-1.06 95% C.I. (1.01, 1.11), and being a female had a lower odd of being in the highest tertile (AOR-0.05 95% C.I. - (0.01, 0.23).

Discussion

In the present study, we compared circulating CAF levels, a potential marker of sarcopenia among study groups with a

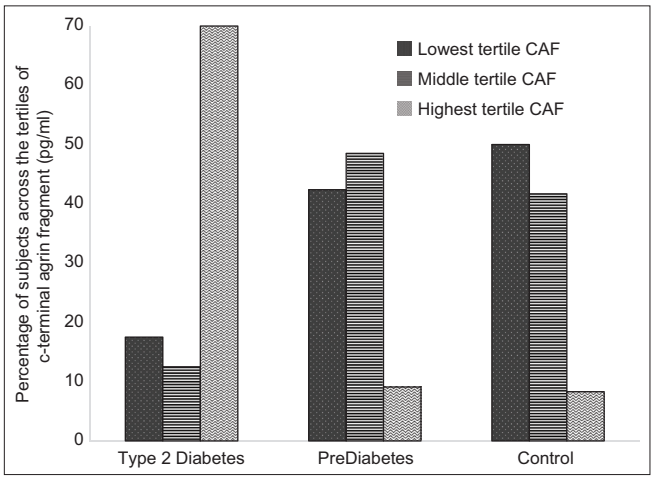

Figure 1: Percentage distribution of the tertiles of circulating C-terminal Agrin fragments levels across the study groups. [Diabetic vs Prediabetic - ($P < 0.001$); Diabetic Vs Control – ($P < 0.001$)]

Table 1: Comparison of physiological, biochemical and muscle strength variables between the study groups

	Controls (*n*=24)	Prediabetes (*n*=33)	Diabetes (*n*=42)	*P*
Age (yrs)[a]	33.9±11.2	35.9±8.9	48.5±6.7	<0.001
Height (m)	1.7±0.1	1.7±0.1	1.6±0.1	<0.001
Weight (kg)	72.5±8.9	72.7±9.9	68.5±11.7	0.168
Body mass index (kg/m^2)	25.5±2.6	25.7±2.6	26.5±4.8	0.432
Body fat (%)	31.3±7.9	32.0±5.2	33.7±9.8	0.462
Fat Mass (kg)	22.3±6.8	22.5±5	22.5±8.2	0.995
Lean body mass (kg)[c]	48.1±6.1	46.2±6.2	43.0±7.5	0.012
Appendicular muscle mass (AMM) (kg)[c]	30.3±4.6	28.3±4.2	25.7±5.5	0.002
Muscle-to-fat ratio	2.4±1.0	2.1±0.5	2.3±1.5	0.632
Biochemistry				
Serum cholesterol (mg/dl)	171.3±40.6	184.8±38.6	171.2±47.5	0.344
HDL (mg/dl)	36.2±7.9	38.8±6.1	37.3±11.9	0.588
LDL (mg/dl)[c]	120.6±30.3	126.7±31.7	105.2±29.8	0.011
Serum triglycerides (mg/dl)[c]	118.1±51.6	136.4±75.1	186.5±141.9	0.028
HbA1c (%)[b]	5.3±0.2	5.8±0.4	8.3±2.1	<0.001
Fasting glucose (mg/dl)[b]	92.0±4.5	97.1±9.3	166.6±65.2	0.019
Basal Insulin (µU/ml)[c]	10.7±5.2	12.3±5.7	15.7±9.1	0.008
HOMA %B[b]	114.5±34.6	111.0±38.2	64.3±45.7	0.005
HOMA-IR[b]	1.43±0.6	1.6±0.7	2.4±1.2	<0.001
Muscle strength				
Muscle quality isometric lower limb (Nm/kg)	3.7±0.9	3.2±0.7	3.3±0.8	0.080
Muscle quality isokinetic lower limb (Nm/kg)	3.0±0.9	2.7±0.7	2.5±0.8	0.057
Sarcopenic index (AMM/Wt)	42.3 (37.2, 45.6)	38.7 (36.8, 40.9)	38.9 (32.3, 41.9)	0.07
Sarcopenic Index (AMM/ht^2)	10.4 (10.0, 11.4)	10.2 (9.1, 10.9)	9.6 (8.5,10.9)	0.08
Muscle strength isometric lower limb[c] (Nm)	112.9±41.0	90.1±22.3	85.6±28.3	0.002
Muscle strength isokinetic lower limb[c] (Nm)	92.7±35.7	76.2±21.2	67.0±26.8	0.002
Serum CAF (pg/ml)[b]	12.3 (9.34, 17.7)	15.6 (8.99, 22.8)	107.6 (23.1, 161.5)	<0.001

[[a]-Groups are significantly different from each other; [b]-DM group is significantly different from the PD and Control group; [c]-DM group is significantly different from the Control group]

Table 2: Factors affecting the circulating CAF levels

	AOR	95% C.I.	*P*
Age (yrs.)	1.06	1.01, 1.11	0.02
Gender (Female)	0.05	0.01, 0.23	<0.0001
BMI (kg/m^2)	0.98	0.85, 1.11	0.77
Muscle strength (Isometric)	0.98	0.97, 1.00	0.08
Study Groups			
Type 2 Diabetes	7.67	2.10, 29.3	<0.01
Pre-diabetes	0.77	0.26, 2.25	0.63

AOR-Adjusted odds ratio; C.I.-Confidence Interval

wide range of glycemic status (PD, T2D and controls). It was observed that the median circulating CAF level among the T2D group was significantly higher compared to other study groups. The circulating CAF was negatively correlated though non-significant with AMM, muscle strength and sarcopenic index. The higher percentage of T2D patients belonged to the higher tertile of CAF values. A notable finding was that after adjusting for age, the T2D group had greater odds (8 times) of falling into the higher tertile of CAF values.

The barriers associated with sarcopenia evaluation impact its diagnosis.[19] Although the Asian working group of Sarcopenia 2019 criteria is available, the adaption of the same to the Indian population might need some consideration. This might be because the muscle mass (one of the criteria for sarcopenia diagnosis) was similar between healthy controls, PD and T2D in a recently published study.[2] Although literature available suggests that there is a strong skeletal muscle atrophy and diabetes association,[20] it might not be true among Indians, at least in terms of muscle mass. Although muscle atrophy might be existing among Indians, as shown by the reduced muscle strength, the accumulation of fat within muscle might be contributing to the maintenance of the muscle mass.[3] This presents a key question: are the present criteria able to unravel sarcopenia among Indians? And is there a need to look for an alternative method along with the present criteria?

The etiology of sarcopenia is multifactorial, and neuromuscular junction (NMJ) detonation is one of the hallmarks.[19] The contributing factors for sarcopenia can either be neurogenic (reduction in motor axon conduction velocity), musculogenic (reduction in motor unit, type of skeletal muscle fibre), synaptogenic (neuromuscular junction degeneration) and/or vasculogenic (changes in microcirculation, the ultrastructure of vascular endothelium). The integrity of NMJ is essential for the functioning of both motor nerves and muscle fibres. Experimental animal studies have shown that impairment of nerve-to-muscle signal transduction leads to

sarcopenia.[20] Markers such as CAF could serve as a biomarker for NMJ disruption resulting in muscle wasting. Motor neurons synthesize agrin, a heparin sulfate proteoglycan, transported along axons and released into synaptic basal lamina of NMJs. Agrin induces assembly of the postsynaptic structures (cluster formation of acetylcholine receptors) and stabilization of presynaptic structures. Agrin is degraded by neurotrypsin to 22-kDa CAF in the NMJ and released into circulation which can be easily measured.[21] Increased circulating CAF levels due to enhanced agrin cleavage indicating ongoing fibre denervation contributing to muscle atrophy and dysfunction could be used as a biomarker for Sarcopenia.[8]

The higher CAF levels noted as part of the present study among the T2D group indicate the onset of sarcopenia. This is in accordance with other studies demonstrating higher plasma CAF22 levels in conditions including chronic obstructive pulmonary disease (COPD) and congestive heart failure patients (CHF).[10] Studies have demonstrated a greater association of serum CAF level with eGFR and proteinuria, predicting a loss of renal function even in the absence of proteinuria in diabetic nephropathy.[11,18,22] The underlying mechanisms though unknown, it has been said that catabolic conditions (CHF, COPD and Chronic Kidney Diseases) disrupt NMJ integrity via proteolytic cleavage of agrin into CAF22 that can be detected and is elevated in circulation.[10] CAF's negative correlation with muscle strength, AMM and sarcopenic index further support that higher CAF signifies poorer skeletal muscle health. The evaluation of the candidate biomarker and its combination with the primary diagnostic criteria (skeletal muscle mass and strength) enhanced the biomarker's diagnostic accuracy in identifying sarcopenia.

The tertile cut-offs of CAF were selected empirically to generate low, medium and high categories. After adjusting for confounding factors such as age and gender, it was seen that T2D patients had almost eight times higher odds of having the highest tertile CAF levels. Skeletal muscle health plays a crucial role both during the onset and progression of T2D.[23] The Korean Genome Epidemiology Study (in 6895 adults with a mean age of 52 years) demonstrated that individuals in the lowest muscle mass index tertile (weight-adjusted appendicular lean mass) had 2-fold higher odds of incident T2D after adjusting for confounders.[24] In addition to the progression of T2D, there is a possibility of increased advanced glycation end-products (AGEs) accumulation, oxidative stress and increased myosteatosis,[25] all interfering with the cellular function of myocytes,[26] potentially leading to the development of sarcopenia.

There are profound health and economic consequences of sarcopenia on the healthcare systems. Confounding factors such as exercise and dietary restriction have been known to rejuvenate the NMJ in catabolic states.[8] The dynamic association between CAF levels and sarcopenia is further elicited by an exercise-induced reduction in plasma CAF levels along with an improvement in the lean body mass in the elderly,[27] and also noted lower levels of CAF in older active dancer compared to their sedentary peers.[28] The study groups of the present study belonged to a sedentary lifestyle. Therefore, the earlier diagnosis along with the implementation of preventive and treatment strategies must gain momentum.

Some of the limitations of the study include: (i) we did not associate circulating CAF levels with some of the other outcomes used to evaluate sarcopenia including gait speed, and so on, as this data were not captured in this cohort.[29] Future studies should explore various outcomes and associations with circulating CAF to expand the utility of the marker in clinical medicine; (ii) the maximum age available as part of the study was 60 years. Comparing T2D above 60 yrs with the existing cohort could have helped us understand the contribution of age and T2D-related changes. Future studies should compare across a wide age range including those above 60 years. This could help represent a wide age range of data related to sarcopenia and diabetes; (iii) A recent study found that combining IL-6, SPARC, MIF and IGF-1 measurements into a single risk score enhanced the diagnostic accuracy compared to single biomarkers, though CAF was not included.[30] In this regard, the diagnostic accuracy of combination biomarker models should be considered, thereby increasing the sensitivity of sarcopenia diagnosis; (iv) The role of hormones in the regulation of muscle mass and strength is well recognized. We did not evaluate the hormonal influence on sarcopenia. However, the lack of studies focusing on the influence of hormones on muscle mass and strength, more so on the circulatory biomarkers in the diagnosis of sarcopenia especially among Indians is needed; (v) The role of medications especially oral hypoglycemic drugs on the development of sarcopenia has been inconclusive.[31] The current study could not explore the association of medications with CAF or muscle outcomes. This was due to the small sample size; statistical exploration was not feasible. The need to explore the impact of medications (oral hypoglycemic drugs) would be one step closer to preventing sarcopenia.

Given the evidence of many studies demonstrating the positive association of serum CAF with sarcopenia, very few studies have not found an association[32] and very few have studied its association with muscle function. These studies have not used gold standard measures of muscle mass or strength to validate CAF as a biomarker, unlike our study which has shown a negative correlation between CAF and skeletal muscle mass, quality and sarcopenic index, all measured by gold standard measures. Nevertheless, the evidence of CAF levels as a screening tool for sarcopenia is still in its infancy requiring more research.

Conclusion

As populations age, the incidence of T2D and sarcopenia tends to increase. Our study demonstrated that patients with T2D have a higher level of CAF than PD and controls. The

8-fold increased odds of CAF levels noted in our study serve as an alert that there could be potential involvement of skeletal muscle among T2D. This is also supported by decreased AMM and muscle strength. Circulating CAF has promising screening utility, hence there is a need to establish a consensus definition for the diagnosis of sarcopenia with CAF as one of the potential measures. This would aid the clinical implementation of treatment and preventive protocols with the use of more targeted exploration into diagnostic biomarkers. The diagnosis of sarcopenia especially among T2D patients is not a routine clinical practice. The main clinical utility of CAF estimation is its potential to be a candidate biomarker for the diagnosis of sarcopenia. The simple serum CAF estimation could potentially translate into clinical practice provided an estimation of CAF is done in a large cohort of T2D Indians. In this direction, the present study is the first Indian study to explore the estimation of CAF levels and compare it with measures of muscle mass and muscle strength.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Key messages

The circulating CAF level has the potential to become a cost-effective routine clinical screening tool for sarcopenia, especially among T2D, and could also assist in planning timely interventions.

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Conflicts of interest

There are no conflicts of interest.

References

1. Marcus RL, Addison O, Dibble LE, Foreman KB, Morrell G, Lastayo P. Intramuscular adipose tissue, sarcopenia, and mobility function in older individuals. J Aging Res 2012;2012. doi: 10.1155/2012/629637.
2. Sucharita S, Harridge SDR, Nidhi S, Sumithra S, Rohatgi P, Kurpad AV. Asian Indians with prediabetes have similar skeletal muscle mass and function to those with Type 2 Diabetes. Front Nutr 2019;6:179.
3. Sucharita S, Pranathi R, Correa M, Keerthana P, Ramesh LJ, Bantwal G, *et al*. Evidence of higher intramyocellular fat among normal and overweight Indians with prediabetes. Eur J Clin Nutr 2019;73:1373-81.
4. Perkisas S, Vandewoude M. "Where frailty meets diabetes." Diab Metab Res Rev 2016;32:261–7.
5. Kim TN, Choi KM. Sarcopenia: Definition, epidemiology, and pathophysiology. J Bone Metab 2013;20:1–10.
6. Cesari M, Fielding RA, Pahor M, Goodpaster B, Hellerstein M, van Kan GA, *et al*. Biomarkers of sarcopenia in clinical trials-recommendations from the International Working Group on Sarcopenia. J Cachexia Sarcopenia Muscle 2012;3:181-90.
7. Marzetti E, Lees HA, Manini TM, Buford TW, Aranda JM Jr, Calvani R, *et al*. Skeletal muscle Apoptotic signaling predicts thigh muscle volume and gait speed in community-dwelling older persons: An exploratory study. PLoS One 2012;7:e32829.
8. Pratt J, De Vito G, Narici M, Boreham C. Neuromuscular junction aging: A role for biomarkers and exercise. J Gerontol A Biol Sci Med Sci 2020. doi: 10.1093/gerona/glaa207.
9. Al-Rubaye AAH, Habeeb AN. Detection of some biomarkers in Sarcopenia patients-Basra city. Eur J Mol Clin Med 2020;7:2416-29.
10. Qaisar R, Karim A, Muhammad T, Shah I, Khan J. Prediction of sarcopenia using a battery of circulating biomarkers. Sci Rep 2021;11:8632.
11. Steubl D, Hettwer S, Vrijbloed W, Dahinden P, Wolf P, Luppa P, *et al*. C-terminal agrin fragment- A new fast biomarker for kidney function in renal transplant recipients. Am J Nephrol 2013;38:501-8.
12. Chamberlain JJ, Rhinehart AS, Shaefer CF Jr, Neuman A. Diagnosis and management of diabetes: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes. Ann Intern Med 2016;164:542–52.
13. Barrett-Connor E. The oral glucose tolerance test, revisited. Eur Heart J 2002;23:1229–31.
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
15. Kuriyan R, Thomas T, Ashok S, Jayakumar J, Kurpad AV. A 4-compartment model-based validation of air displacement plethysmography, dual energy X-ray absorptiometry, skinfold technique and bio-electrical impedance for measuring body fat in Indian adults. Ind J Med Res 2014;139:700–7.
16. Kwon HJ, Ha YC, Park HM. The reference value of skeletal muscle mass index for defining the Sarcopenia of women in Korea. J Bone Metab 2015;22:71–5.
17. Metter EJ, Lynch N, Conwit R, Lindle R, Tobin J, Hurley B. Muscle quality and age: Cross-sectional and longitudinal comparisons. J Gerontol Biol Sci 1999;54A: B207–18.
18. Devetzis V, Daryadel A, Roumeliotis S, Theodoridis M, Wagner C, Hettwer S, *et al*. C-Terminal Fragment of Agrin (CAF): A novel marker for progression of kidney disease in type 2 diabetes. PLoS One 2015;10:e0143524.
19. Cruz-Jentoff 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.
20. Perry BD, Caldow MK, Brennan-Speranza TC, Sbaraglia M, Jerums G, Garnham A, *et al*. Muscle atrophy in patients with Type 2 diabetes mellitus: Roles of inflammatory pathways, physical activity and exercise. Exerc Immunol Rev 2016;22:94-109.
21. Butikofer L, Zurlinden A, Bolliger MF, Kunz B, Sonderegger P. Destabilization of the neuromuscular junction by proteolytic cleavage of agrin results in precocious sarcopenia. J FASEB 2011;25:4378-93.
22. Steubl D, Roos M, Hettwer S, Satanovskij R, Tholen S, Wen M, *et al*. Plasma total C-terminal agrin fragment (tCAF) as a marker for kidney function in patients with chronic kidney disease. Clin Chem Lab Med 2016;54:1487-95.
23. Defronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in Type 2 diabetes. Diab Care 2009;32:S157-63.
24. Hong S, Chang Y, Jung HS, Yun KE, Shin H, Ryu S. Relative muscle mass and the risk of incident type 2 diabetes: A cohort study. PLoS One 2017;12:e0188650.
25. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules 2015;5:194–222.
26. Scott D, de Courten B, Ebeling PR. Sarcopenia: A potential cause and consequence of type 2 diabetes in Australia's ageing population? Med J Aust 2016;205:329–33.
27. Drey M, Sieber CC, Bauer JM, Uter W, Dahinden P, Fariello RG, *et al*. C-terminal Agrin Fragment as a potential marker for sarcopenia

caused by degeneration of the neuromuscular junction. Exp Gerontol 2013;48:76–80.
28. Marcolin J, Franchi MV, Monti E, Pizzichemi M, Sarto F, Sirago G, *et al*. Active older dancers have lower C-terminal agrin fragment concentration, better balance and gait performance than sedentary peers. Exp Gerontol 2021;153:111469.
29. Pesola GR, Terla V, Pradhan M. Advanced skeletal muscle mass reduction (Sarcopenia) secondary to neuromuscular disease. Case Rep Crit Care 2020;2020. doi: 10.1155/2020/8834542.
30. Kwak JY, Hwang H, Kim SK, Choi JY, Lee SM, Bang H, *et al*. Prediction of sarcopenia using a combination of multiple serum biomarkers. Sci Rep 2018;8:8574. doi: 10.1038/s41598-018-26617-9.
31. Stangl MK, Bocker W, Ferrari U, Chubanov V, Fischereder M, Gudermann T, *et al*. Sarcopenia-Endocrinological and neurological aspects. Exp Clin Endocrinol Diabetes 2019;127:8–22.
32. Hester GM, VanDusseldorp TA, Ha PL, Kiani K, Olmos AA, Jabbari M, *et al*. Microbiopsy sampling for examining age-differences in skeletal muscle fiber morphology and composition. Front Physiol 2022;12:756626.