DETAILS OF RESEARCH WORK DULY SIGNED BY APPLICANT

1. Summary of research work

I, Dr. Rimesh Pal, along with my team had conducted a community-based epidemiological survey in the Union Territory of Chandigarh. The survey had been named Chandigarh Urban Bone Epidemiological Study (Acronym: CUBES). As a part of CUBES, we had recruited more than 900 ostensibly healthy adults through a pre-determined set of inclusion and exclusion criteria. All the included subjects had undergone an array of blood investigations; in addition, all had undergone radiological investigations like dual-energy X-ray absorptiometry (DEXA) for assessment of bone mineral density (BMD) and body composition (notably muscle and fat mass). Besides, all underwent estimation of handgrip strength (surrogate of muscle strength) and gait speed (surrogate of physical performance). The database, hence created, had been used to generate Indian normative data on metabolic bone profile (serum calcium, phosphate, alkaline phosphatase), bone mineral density, bone turnover markers and muscle mass/muscle strength. The data was used to generate indigenous cut-offs to define low muscle strength and low muscle mass in Indian adults; using these cut-offs, we were able to find out the prevalence of sarcopenia and sarcopenic obesity in the Indian community.

2. Idea behind conducting this survey

India is home to more than 1.4 billion people. A significant proportion of the Indian population is comprised by the elderly (≥ 65 years). As per the 2011 census, elderly accounted for 9% of the Indian total population and the number is expected to rise to 18% by 2036. With improved healthcare facilities, the average life expectancy of the Indian population has increased, thereby, apparently resulting in a boom in the

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number of the elderly individuals. Nevertheless, the elderly are prone to develop multiple health-related disorders; osteoporosis, sarcopenia and sarcopenia obesity are entities that are much more common in the elderly population.

Osteoporosis, sarcopenia and sarcopenic obesity are all associated with an increased risk of morbidity, frequent hospitalizations, poor quality of life and eventually mortality. However, if detected early and treated, the morbidity and mortality resulting out of osteoporosis and sarcopenia can be reduced. Hence, the cornerstone remains early detection.

The diagnosis of osteoporosis is based on the demonstration of low BMD T-scores as measured using DEXA scanners. However, the DEXA scanners that are used worldwide including those being used in India generate BMD T-scores based on an in-built reference database, which is derived from the Caucasian population (NHANES data). It is very well known that Indians have 5-10% lower BMD than Caucasian counterparts. So, using a higher normative range for diagnosing osteoporosis in Indians seems unwise and illogical. This can inadvertently lead to over-diagnosis of osteoporosis/osteopenia and can lead to initiation of anti-osteoporotic therapy in otherwise healthy subjects who might have been identified to have osteoporosis based on the Western reference database. Such unnecessary treatments can increase the financial burden on the country and thereby lead to wastage of medical resources.

Similarly, sarcopenia and its counterpart, sarcopenic obesity is not uncommon in the elderly population. Sarcopenia is characterized by low muscle strength and low muscle mass that eventually results in poor physical performance. On the other hand, sarcopenic obesity is characterized by the co-occurrence of sarcopenia and obesity in the same individual; the presence of sarcopenic obesity portends a poorer prognosis

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than sarcopenia or obesity alone. In spite of their implications of health, sarcopenia and sarcopenic obesity remains an under-recognized and under-reported entity with lack of awareness about the same amongst the masses and physicians alike. In fact, till 2020, there had been no robust data on the prevalence of sarcopenia or sarcopenic obesity from India.

The diagnosis of sarcopenia relies on the demonstration of low muscle strength and low muscle mass; however, as with BMD, Indians, by virtue of their built, have lower muscle strength and muscle mass compared to their Caucasian counterparts. Thus, here again, using well-established Caucasian cut-offs to define sarcopenia or sarcopenic obesity would be unwise.

The management of osteoporosis entails use of certain anti-osteoporotic therapy which can be broadly classified into 2 categories: anti-resorptive agents and osteoanabolic agents. Anti-osteoporotic therapy often requires long-term treatments with regular monitoring of treatment response using periodic BMD measurements. Nevertheless, changes in BMD with anti-osteoporotic therapies require at least 1 year and BMD does not help assess treatment response in the short term. To circumvent this problem, in clinical practice as well as in research, certain circulating proteins called bone turnover markers (BTMs) are monitored as a surrogate marker of treatment response to anti-osteoporotic therapies.

Several global osteoporosis guidelines recommend the use of serum type I collagen C-telopeptide (CTX) and serum procollagen type I N-propeptide (PINP) for monitoring the response and assessing the adherence to osteoporosis treatment. Nevertheless, BTMs exhibit marked inter-ethnic variation; hence, population-specific data are required. However, such population specific data had been missing from our country till date.

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Considering the noticeable paucity of normative data from the Indian population, we planned to conduct a community-based epidemiological survey to generate normative data on metabolic bone profile parameters (like serum calcium, phosphate, alkaline phosphatase), BMD, PINP, CTX, muscle mass and muscle strength and thereafter determine the prevalence of osteoporosis/osteopenia and sarcopenia/sarcopenic obesity in the recruited population.

3. Brief methodology

Apparently healthy individuals aged 20 years and above were recruited in the study. Individuals were chosen from four sectors of Chandigarh; these sectors were in turn selected by simple random sampling. Houses within each sector were chosen by systematic random sampling, selecting every fifth house from a random starting point. Household members eligible for enrollment were selected using the 'Kish Selection Method'. A pre-prepared proforma comprising of questions on demography, menstrual history, co-morbidities, addictions, and drug intake was filled for the selected individual. In addition, a dietary history was obtained using the 24-h dietary recall method and physical activity assessed using the Global Physical Activity Questionnaire (GPAQ). Participants eligible for the study underwent blood sampling after an overnight fast. Laboratory investigations included hemoglobin, creatinine, bilirubin, alanine transaminase, aspartate transaminase, serum albumin, total calcium, inorganic phosphorous, alkaline phosphatase, fasting blood glucose, glycated hemoglobin, testosterone, thyroid function test, 25-hydroxyvitamin D, intact parathyroid hormone, and IgA tissue transglutaminsase [IgA tTg] antibody. Volunteers without obvious biochemical abnormalities underwent dual-energy X-ray absorptiometry (DEXA) scan using the HOLOGIC Discovery A (QDR 4500;

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Hologic, Inc., Bedford, MA) scanner for assessment of body composition. All DEXA scans were performed by a dedicated, International Society of Clinical Densitometry (ISCD)-certifed technician. Quality control procedures were carried out in accordance with the manufacturer's recommendations. A thorough physical examination and anthropometry was performed prior to DEXA scan.

Handgrip strength was measured in each participant using the Jamar Plus Digital Hand Dynamometer (Jamar®, Patterson Medical). Usual gait speed was measured using a 4-min walk test. A distance of 4 m was marked on the floor. The study participants were advised to walk at a comfortable speed from the start point to the stop point. The time taken to traverse the distance was measured using a stopwatch. Each participant was given two walking trials and the average of the two gait speeds (in m/s) was considered for final analysis.

Inclusion criteria comprised of all participants chosen by 'Kish Selection Method' who were willing to provide written informed consent for the study. Exclusion criteria were exhaustive and were applicable at each and every stage of the survey: (a) At the time of initial proforma filling, individuals with a history of hepatic, renal, neoplastic, respiratory, rheumatological, gastrointestinal, dermatological, endocrine (notably diabetes mellitus, hyperthyroidism, Cushing's syndrome, hyperparathyroidism), systemic infective disorders, chronic drug intake (especially steroids, statins, complementary and alternative medications, and protein supplements), addictions, total calorie intake < 2100 kcal/day (minimum required calorie level for a healthy and active life as proposed by Indian Council of Medical Research), physical inactivity (defined as < 250 MET-minutes/week), and contraindications to DEXA scan (pregnancy, implant placement) were excluded at the outset; (b) Following biochemical investigations, participants identified as having anemia (as per WHO

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definition), renal dysfunction (estimated glomerular filtration rate calculated by CKDEPI formula < 90 ml/min/1.73 m^2), low serum albumin (< 3.5 gm/dl), diabetes mellitus (as per ADA definition), hyperthyroidism (subclinical or overt), overt hypothyroidism, hypercalcemia, 25-hydroxyvitamin D < 10 ng/ml) were excluded prior to DEXA scan. Participants with pre-diabetes and subclinical hypothyroidism were, however, not excluded; (c) Following anthropometry, participants with a BMI < 18.5 kg/m^2 were excluded.

All included participants also underwent estimation of serum total PINP and β-CTX.

4. Statistical Analysis

Sample size was calculated using the formula $n = 4pq/l^2$, where p=population proportion of low muscle mass, q=1-p, and L=allowable error. For this study, L was presumed to be 20% of p giving a power of (1-L), i.e., 80% to study. p was taken as 15% based on the prevalence of low muscle mass in females according to the study of Marwaha et al. This yielded a value of 567. Keeping in mind the 'design effect', we had applied a correction factor of 1.2, and thus, the sample size after correction came out to be 680. The final sample size was kept as 750 to account for non-responders. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) 23.0 software program (SPSS Inc., Chicago, IL, USA). Normality of data was checked using Kolmogorov–Smirnov test. Muscle mass was assessed using appendicular skeletal muscle mass index (ASMI) which was calculated as the sum of lean mass at arms and legs in kilograms divided by square of height in meters. Muscle strength was expressed in terms of dominant handgrip strength. All participants were divided into 10-year age groups. Appendicular skeletal muscle index, dominant handgrip, and usual gait speed in each age group were reported as mean ± standard

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deviation (SD). Comparisons of HGS, ASMI, and GS between males and females were made using Independent-Student T Test. HGS and ASMI of the study participants were compared with Caucasian counterpart using well-established NHANES data. For the purpose of generating cut-offs, individuals belonging to the 20–39 years age group were considered as the young reference population. Based on the EWGSOP recommendation, cut-of for low muscle strength was defined as dominant HGS two SD below the mean of young reference population. Similarly, low muscle mass was defined as ASMI two SD below the mean of young reference population. Cut-offs were sex-specifc. Usual gait speed≤0.8 m/s defined low physical performance.

Based on these cut-offs, the prevalence of probable sarcopenia, sarcopenia, and severe sarcopenia was estimated. Likewise, prevalence was established based on the Western (as mentioned in the EWGSOP 2010 consensus statement), EWGSOP2 (2018), International Working Group on Sarcopenia (IWGS), and Asian Working Group for Sarcopenia (AWGS 2014 and 2019) guidelines. Finally, correlations between biochemical parameters and components of sarcopenia were made using Pearson/ Spearman correlation followed by multiple linear regression analysis with backward elimination.

5. Results

After exclusion, 804 participants were included in the final analysis. The group included 339 male participants (M:F=1.13:1.55). Out of the 465 female participants, 302 were premenopausal. The mean age of the group was 44.4±15.4 years (range 20–85 years). There was no significant difference in age between males and females (p=0.548).

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Subsequent data has been presented in the form of tables or graphs:

Table 1 Biochemical parameters of the study participants ($N=804$)

Biochemical parameter	Value
Serum albumin (mean ± SD) (gm/dl)	4.37 ± 0.21
Serum corrected calcium (mean ± SD) (mg/dl)	9.12 ± 0.16
Serum alkaline phosphatase [median (IQR)] (IU/l)	102.0 (72.0–117.0)
Serum iPTH [median (IQR)] (pg/ml)	45.98 (32.73–62.46)
Serum 25-hydroxyvitamin D [median (IQR)] (ng/ml)	16.19 (12.40–26.07)
Serum testosterone [median (IQR)] (nmol/l)	
Male	16.09 (13.40–19.47)
Female	0.87 (0.46–1.26)

SD standard deviation, *IQR* interquartile range, *iPTH* intact parathyroid hormone

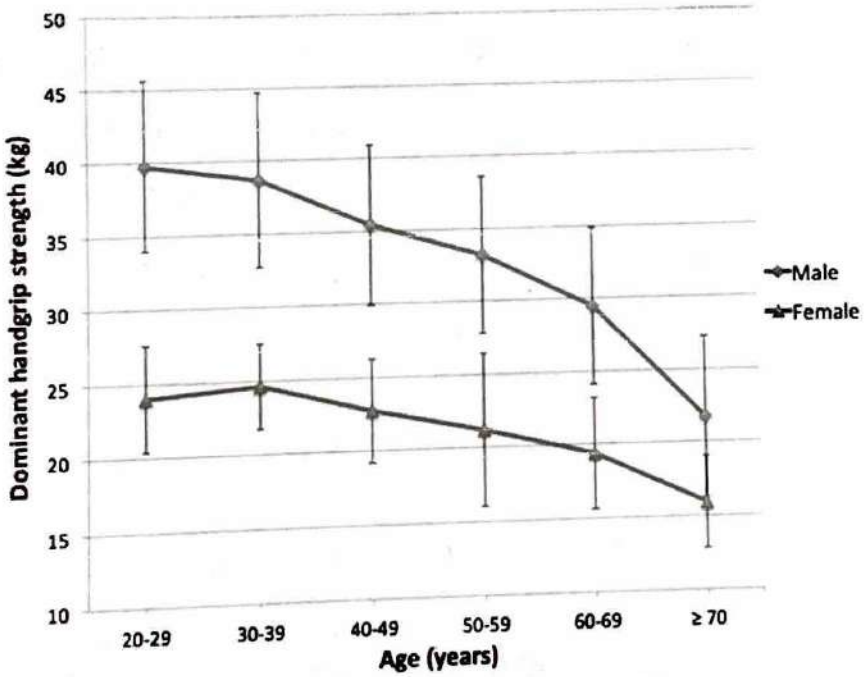

Figure 1. Line diagram showing decade-wise distribution of dominant handgrip strength (in kg) in males and females

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Table 2 Comparison of dominant handgrip strength (HGS, in kg) between Caucasians (obtained from NHANES data) and study participants

Age group	Males			Females		
	NHANES dominant HGS (kg)	Present study cohort dominant HGS (kg)	Percent difference	NHANES dominant HGS (kg)	Present study cohort dominant HGS (kg)	Percent difference
20–25	47.3	38.6	− 18.4	30.5	23.4	− 23.3
25–30	48.6	40.6	− 16.5	31.1	24.5	− 21.2
30–35	50.3	39.2	− 22.1	31.6	24.9	− 21.2
35–40	49.4	38.3	− 22.5	31.2	24.4	− 21.8
40–45	48.1	36.4	− 24.3	30.8	23.1	− 25.0
45–50	47.4	34.5	− 27.2	30.9	22.5	− 27.2
50–55	44.4	33.7	− 24.1	29.2	22.4	− 23.3
55–60	43.8	32.7	− 25.3	28.6	21.3	− 25.5
60–65	41.3	29.6	− 28.3	27.1	19.7	− 27.3
65–70	40.1	29.5	− 26.4	25.7	19.2	− 25.3
70–75	38.8	27.2	− 29.9	24.0	16.4	− 31.7
75–80	35.3	21.6	− 38.8	23.1	15.8	− 31.6
80–85	31.4	16.7	− 46.8	19.5	12.3	− 36.9

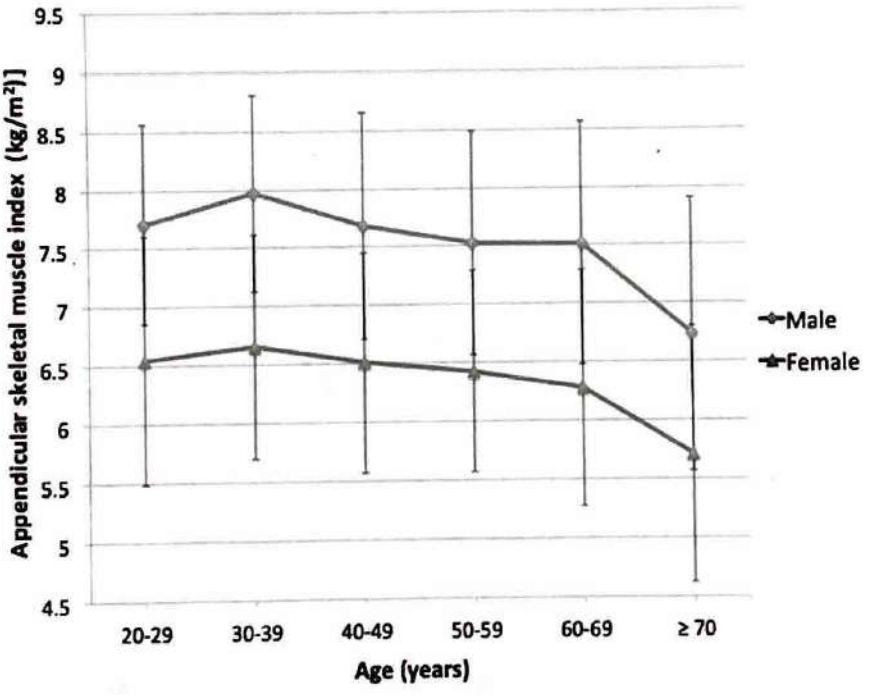

Figure 2. Line diagram showing decade-wise distribution of appendicular skeletal muscle index (in kg/m^2) in males and females

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Table 3 Comparison of appendicular skeletal muscle index (ASMI, in kg/m^2) between Caucasians (obtained from NHANES data) and study participants

Age group	Males			Females		
	NHANES (White) ASMI (in kg/m^2)	Present study cohort ASMI (in kg/m^2)	Percent difference	NHANES (White) ASMI (in kg/m^2)	Present study cohort ASMI (in kg/m^2)	Percent difference
20–25	8.87	7.71	− 15.0	6.81	6.49	− 4.9
25–30	8.94	7.79	− 14.8	6.86	6.58	− 4.3
30–35	9.02	8.10	− 11.4	6.90	6.68	− 3.3
35–40	9.09	7.96	− 14.2	6.93	6.62	− 4.7
40–45	9.12	7.70	− 18.4	6.95	6.53	− 6.4
45–50	9.11	7.62	− 19.6	6.93	6.46	− 7.3
50–55	9.05	7.58	− 19.4	6.90	6.44	− 7.1
55–60	8.95	7.53	− 18.9	6.84	6.38	− 7.2
60–65	8.81	7.52	− 17.2	6.76	6.31	− 7.1
65–70	8.64	7.48	− 15.5	6.67	6.16	− 8.3
70–75	8.44	7.28	− 15.9	6.57	6.02	− 9.1
75–80	8.21	6.51	− 26.1	6.45	4.57	− 41.1
80–85	7.97	4.72	− 68.9	6.33	4.72	− 34.1

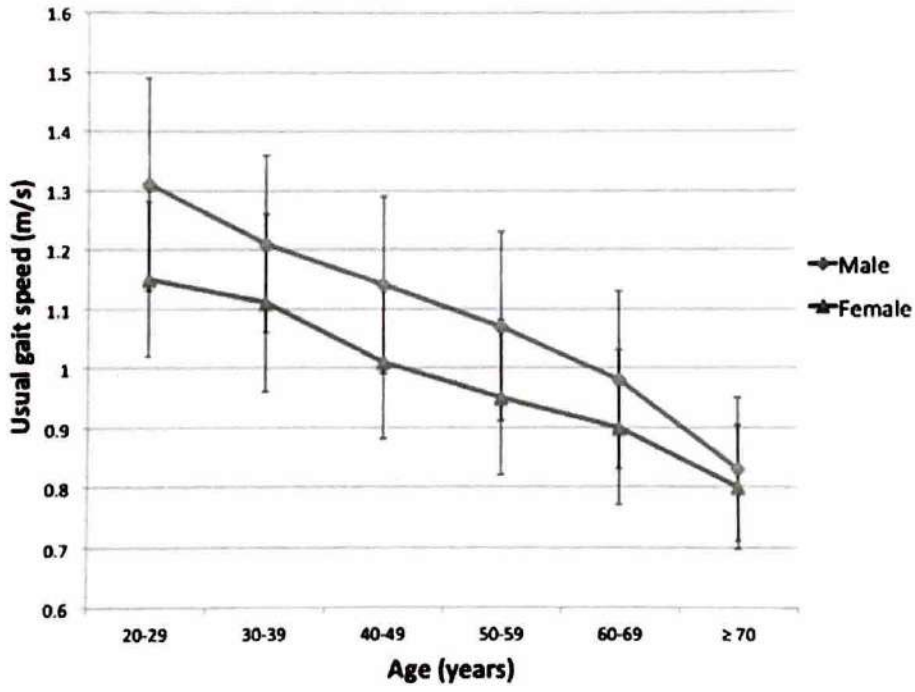

Figure 3. Line diagram showing decade-wise distribution of usual gait speed (in m/sec) in males and females

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Table 4 Comparison of cut-offs to define low muscle strength and low muscle mass derived from the present study with those proposed by international organizations

	Present study		EWGSOP (2010)		IWGS[c]		EWGSOP2 (2018)		AWGS (2014)		AWGS (2019)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Dominant handgrip strength (in kg)	27.5	18.0	30.0[a]	20.0[a]	–	–	27.0	16.0	26.0	18.0	28.0	18.0
ASMI (in kg/m^2)	6.11	4.61	7.26[b]	5.50[b]	7.23	5.67	7.00	5.50	7.00	5.40	7.00	5.40

EWGSOP European Working Group on Sarcopenia In Older People, *IWGS* International Working Group on Sarcopenia, *AWGS* Asian Working Group for Sarcopenia, *ASMI* Appendicular skeletal muscle index

[a]Dominant handgrip strength cut-offs mentioned in the EWGSOP 2010 guidelines based on the study by Lauretani et al. [24]

[b]ASMI cut-offs mentioned in the EWGSOP 2010 guidelines based on the Rosetta study [23]

[c]IWGS does not propose cut-offs to define poor muscle strength [25]

	Present study	EWGSOP (2010)	EWGSOP2 (2018)	AWGS (2014)	AWGS (2019)
Probable sarcopenia					
Male	57 (16.8%)	87 (25.6%)	55 (16.2%)	42 (12.3%)	59 (17.4%)
Female	61 (13.1%)	127 (27.3%)	24 (5.2%)	61 (13.1%)	61 (13.1%)
Total	118 (14.6%)	214 (26.6%)	79 (9.8%)	103 (12.8%)	120 (14.9%)
Sarcopenia					
Male	16 (4.7%)	55 (16.2%)	35 (10.3%)	29 (8.5%)	29 (8.5%)
Female	10 (2.1%)	39 (8.4%)	07 (1.5%)	25 (5.3%)	25 (5.3%)
Total	26 (3.2%)	94 (11.6%)	42 (5.2%)	54 (6.7%)	54 (6.7%)

EWGSOP European Working Group on Sarcopenia In Older People, *AWGS* Asian Working Group for Sarcopenia

6. DISCUSSION AND CONCLUSION

In this study, we have generated indigenous cut-offs to define low muscle strength and low muscle mass, and have estimated the prevalence of probable sarcopenia, sarcopenia, and severe sarcopenia in a population of ostensibly healthy adults randomly recruited from the community by door-to-door survey. We have demonstrated that muscle mass (expressed as appendicular skeletal muscle index) is lower in Indians compared to Caucasians across all decades, hence, applying Western cut-offs leads to an undue overestimation of sarcopenia in the Indian context. Using

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the cut-offs derived from the present study, we found that the prevalence of 'probable sarcopenia', 'sarcopenia', and 'severe sarcopenia' in the community were 14.6%, 3.2%, and 2.3%, respectively, figures that seem to be more realistic. Considering the dearth of data on sarcopenia from the Indian subcontinent, we believe that the present study will pave the way for large-scale observational and interventional studies from this part of the world.

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