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An index of oral-type gut genera is associated with lower muscle mass and with 3- year muscle loss in community-dwelling older adults: a cohort study

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Abstract

Background

Evidence on the gut-muscle axis is mostly cross-sectional and framed around microbial diversity. We examined whether a prespecified index of oral-type gut genera — organisms normally resident in the mouth whose faecal appearance marks a failing gastric acid barrier — was associated with muscle mass, muscle function and subsequent 3-year muscle loss.

Methods

In the Kyotango Longevity Cohort Study, 782 community-dwelling adults aged 65 or older had faecal 16S rRNA profiling and a muscle assessment; 493 were reassessed a mean of 3.35 years later. Exposures were alpha diversity, community composition, 130 genera, and an index prespecified as the mean standardised centred log-ratio abundance of Streptococcus, Veillonella, Fusobacterium, Granulicatella, Actinomyces, Rothia and Haemophilus. Appendicular skeletal muscle mass (ASM) was the primary outcome; ASM/height², ASM/BMI, grip strength, knee extension strength and gait speed were secondary, with the false discovery rate controlled across them and across change in each at follow-up. Covariates were entered in five prespecified blocks, ending with gastrointestinal medication, constipation and disease. Longitudinal models were repeated with censoring weights.

Results

Per 1 SD of the index, ASM was lower by 0.075 SD (95% CI 0.034 to 0.116) adjusted for age, sex and survey year, and by 0.067 SD (0.029 to 0.105) fully adjusted. Every secondary outcome survived correction except grip strength (q = 0.102): ASM/BMI − 0.059, gait speed − 0.088, ASM/height² -0.046 and knee extension − 0.067 (all q ≤ 0.026). Annual change in ASM was − 0.125%/year (-0.235 to -0.015) fully adjusted and − 0.180%/year (-0.292 to -0.067) weighted, but change in grip strength, knee extension strength and gait speed was unrelated to the index (q = 0.36 to 0.88), as was change in every diversity measure. Proton pump inhibitor use raised the index by 0.49 SD (0.18 to 0.81); no inflammatory marker was associated with it.

Conclusions

An index of oral-type gut genera was associated with lower muscle mass, weaker knee extension and slower gait, and with subsequent loss of muscle mass but not of strength or speed; diversity was associated with none of these. The associations are small and observational. The gut-muscle axis may be better studied at taxonomic resolution and against continuous measures.

Background

Sarcopenia predicts disability, falls and mortality, yet its determinants beyond age, nutrition and physical activity remain unclear. Attention has turned to the gut microbiota, on the premise that microbial products reach skeletal muscle through the circulation and that age-related changes in the community contribute to muscle loss, the gut-muscle axis [1, 2]. Lactobacillus casei attenuates age-related muscle decline in mice [3], and probiotics have been proposed as therapy [4]. Most human evidence, however, is cross-sectional, based on small clinical samples, and framed around diversity, which is assumed to index a healthy community [5].

Two problems limit such studies. First, diversity is a single summary of an entire community, and a summary insensitive to the biology of interest returns null results even when specific organisms matter [1]. Second, without repeated measurement a microbial signature preceding muscle loss cannot be distinguished from one following it.

One group of organisms has a prior rationale that does not depend on the data. The genera normally resident in the oral cavity and upper gastrointestinal tract, among them Streptococcus, Veillonella, Fusobacterium, Granulicatella, Actinomyces, Rothia and Haemophilus, are continuously swallowed and ordinarily eliminated by gastric acid. Their appearance in faeces marks failure of that barrier, rises with proton pump inhibitor use [6, 7], and drives intestinal T-cell responses and inflammation in experimental models [8]. Because chronic low-grade inflammation contributes to muscle loss, this group is a biologically rather than statistically selected candidate. We refer to these organisms as oral-type genera, a descriptive term for taxa characteristically abundant in the oral cavity; the term is operational and is not a claim that the faecal sequences originated in the mouth of the individual concerned.

Two further considerations shaped this analysis. The Asian Working Group for Sarcopenia has issued updated 2025 criteria [9], so associations require re-examination under the revised definition rather than assumed carry-over from the 2019 consensus [10]. Grip strength has dominated this literature, whereas lower-limb strength and gait speed are more relevant to mobility; whether the microbiota relates to them as it does to grip is unknown.

We therefore examined, in a population-based cohort of older Japanese adults, whether an index of oral-type gut genera was associated with muscle mass and function, and whether it was associated with subsequent 3-year change in muscle mass. Diversity, community composition and individual genera were examined alongside the index.

Methods

Study design and participants

The Kyotango Longevity Cohort Study enrols community-dwelling residents aged 65 years or older in Kyotango City, Kyoto Prefecture, Japan, a rural region with a high proportion of long-lived residents [11,

12]. The present report has two components, which are kept distinct throughout.

The cross-sectional component comprises the 782 participants who had a baseline faecal sample and a baseline muscle assessment, enrolled between 2017 and 2021. Its question is whether microbial features are associated with concurrent muscle mass and function. The longitudinal component comprises the 493 of those participants who attended a second examination, and its question is whether baseline microbial features are associated with subsequent change in muscle mass. A third, exploratory component comprises the 160 participants who also had a dental examination. The longitudinal sample is smaller and differs systematically from the full cohort, so estimates from the two components are not directly comparable; the comparison of participants with and without reassessment, and the weighting used to address it, are described below.

Study size was determined by cohort attendance and no a priori sample size calculation was performed. The study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine (ERB-C-885-10) and all participants gave written informed consent.

Faecal sampling, DNA extraction and sequencing

Stool samples were collected at home at the baseline examination, before the clinical examination and without any change to the participant's usual diet. Faecal samples the size of a rice grain were collected into guanidine thiocyanate solution (Feces Collection kit, Techno Suruga Lab, Shizuoka, Japan). After vigorous mixing, samples were stored at room temperature or below for no more than 7 days until DNA extraction. Faecal microbial DNA was extracted with the Maxwell RSC Fecal Microbiome DNA Kit (Promega, Tokyo, Japan) according to the manufacturer's instructions.

The V3-V4 region of the 16S rRNA gene was amplified with the primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') and Tks Gflex DNA Polymerase (TaKaRa Bio, Kusatsu, Japan). Thermal cycling comprised an initial denaturation at 95 C for 3 min, 25 cycles of 95 C for 30 s, 55 C for 30 s and 72 C for 30 s, and a final elongation at 72 C for 5 min. Amplicons were purified on NucleoFast 96 PCR plates (TaKaRa Bio), and indexing polymerase chain reaction was performed with unique dual-index primer sets compatible with MiSeq sequencing (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. Indexed products were purified, normalised with the SequalPrep Normalization Plate Kit (Life Technologies, Tokyo, Japan) and pooled at equimolar concentration. The pooled library was purified with AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) and sequenced as 285 bp paired-end reads on the Illumina MiSeq platform with the MiSeq Reagent Kit v3.

Sequencing data were processed in QIIME 2 version 2024.5 [13, 14]. Demultiplexed paired-end reads were imported in PairedEndFastqManifestPhred33 format. Quality filtering, read merging, chimaera removal and amplicon sequence variant (ASV) inference were performed with the q2-dada2 plugin (dada2 denoise-paired); the first 17 and 19 bases were trimmed from the forward and reverse reads

respectively, and the reads were truncated at 260 and 240 bases respectively. Taxonomy was assigned with the q2-feature-classifier plugin (feature-classifier classify-sklearn) against a SILVA 138 reference classifier clustered at 99% sequence identity [15]. ASVs with a total frequency below two reads across the dataset were removed with q2-feature-table (feature-table filter-features, minimum frequency 2). Representative ASV sequences were placed phylogenetically with q2-fragment-insertion (fragment-insertion sepp) against the SILVA 128 SEPP reference database, and ASVs that could not be placed were excluded. Features classified as mitochondria or chloroplasts were removed with q2-taxa (taxa filter-table), and only features with a phylum-level assignment were retained. The filtered feature table was collapsed to genus level with q2-taxa (taxa collapse, level 6) and relative abundances were calculated with q2-feature-table (feature-table relative-frequency). Default parameters of QIIME 2 version 2024.5 were used unless stated otherwise. The median read depth of the final genus-level feature table was 84,427 reads per sample (interquartile range 61,700 to 113,036; range 21,105 to 405,546), and no sample fell below 21,000 reads. Samples were not rarefied to a common depth, so alpha diversity was calculated on the unrarefied table; because richness measures depend on sequencing depth, all analyses involving diversity were repeated with the base-10 logarithm of read depth as an additional covariate. The sequencing identifier of every participant, with age, sex and body mass index, is listed in Additional file 1: Table S1.

Microbial exposures

Four microbial exposures were defined in advance, and all were derived from the baseline sample, which was the only faecal sample collected.

Of the 432 genus-level features detected, 130 genera were retained for analysis: those present in at least 10% of participants and with a mean relative abundance of at least 0.01%. Genus-level assignment accounted for a mean of 94.0% of the relative abundance per sample (range 69.6% to 99.8%), and the 130 retained genera for a mean of 93.2% (range 66.5% to 99.5%); the remainder were features that could not be resolved below family level, which were excluded from the genus-level analyses.

Alpha diversity was summarised by observed genus richness, which ranged from 47 to 397, the Shannon index and Chao1. Community composition was represented by the Aitchison distance, the Euclidean distance between centred log-ratio (CLR) transformed genus abundances. For the CLR transformation, zero counts were replaced by a pseudocount of 1.0×10^{-6} and the transformation was applied within each sample relative to that sample's geometric mean; the resulting values were then standardised across participants to mean 0 and SD 1 (CLR-Z), so that genus-level coefficients are expressed per 1 SD. Individual genera were the 130 retained genera in CLR-Z form. The oral-type genera index was the mean of the CLR-Z values of the seven genera named in the Background, itself standardised to mean 0 and SD 1.

The index was specified before any analysis of these data, on the biological grounds set out in the Background and without reference to associations with muscle. Its seven constituents were weighted

equally; no data-driven weighting was applied. Internal consistency was acceptable (Cronbach's alpha 0.818; mean pairwise correlation between constituents 0.391; first principal component 48.5% of variance). Sensitivity of the index to each constituent was assessed by recomputing it seven times, each time omitting one genus.

Muscle outcomes

Appendicular skeletal muscle mass (ASM) was measured by multifrequency bioelectrical impedance (InBody770; InBody Japan, Tokyo, Japan) and expressed as ASM/height² and ASM/BMI, the two indices permitted by AWGS 2025 [9], with low muscle mass defined by the AWGS bioelectrical impedance cut-off points; the agreement between the two indices in this cohort is reported separately. Grip strength was measured with a hydraulic dynamometer (Saehan SH5001, SAKAImed, Tokyo, Japan) as the maximum of both hands. Isometric knee extension strength was measured with a handheld dynamometer (micro-TAS F-1, ANIMA, Tokyo, Japan) and analysed as absolute force, per body weight and per leg lean mass. Knee flexion and maximum gait speed were recorded at the same session. Sarcopenia was defined by AWGS 2025 as low muscle mass by either index together with low grip strength, below 28 kg in men and 18 kg in women. Height and weight were measured by the same bioelectrical impedance device at the same examination, and body mass index was derived from them; this is the body mass index used for the ASM/BMI index, for Table 1 and for every model. All continuous muscle outcomes were standardised to mean 0 and SD 1, so that coefficients are expressed as the SD change in the outcome per 1 SD of the exposure.

Table 1
Characteristics of the 782 participants across tertiles of the oral-type genera index

Characteristic	Tertile 1 (n = 261)	Tertile 2 (n = 260)	Tertile 3 (n = 261)	P	P for trend
Demographic and anthropometric					
Age, years	72.5 ± 5.8	72.9 ± 5.2	73.3 ± 5.9	0.268	0.105
Men, n (%)	87 (33.3)	102 (39.2)	126 (48.3)	0.002	< 0.001
Body mass index, kg/m²	23.1 ± 2.9	22.9 ± 3.1	23.1 ± 3.1	0.576	0.795
Muscle mass, strength and performance					
Appendicular skeletal muscle mass, kg	15.94 ± 3.77	16.01 ± 3.76	16.23 ± 3.77	0.661	0.384
ASM/height², kg/m²	6.43 ± 0.93	6.43 ± 0.95	6.52 ± 0.94	0.461	0.267
ASM/BMI	0.693 ± 0.157	0.705 ± 0.155	0.710 ± 0.161	0.473	0.233
Low ASM/height², n (%)	85 (32.6)	95 (36.5)	93 (35.6)	0.608	0.463
Low ASM/BMI, n (%)	87 (33.3)	102 (39.2)	116 (44.4)	0.034	0.009
Low muscle mass by either index, n (%)	128 (49.0)	151 (58.1)	164 (62.8)	0.005	0.002
Grip strength, kg	28.8 ± 8.6	28.6 ± 8.0	29.7 ± 8.6	0.254	0.182
Low grip strength, n (%)	22 (8.4)	16 (6.2)	22 (8.4)	0.530	1.000
Knee extension strength, kg	27.9 ± 9.4	28.0 ± 9.5	27.7 ± 9.4	0.951	0.810
Maximum gait speed, m/s	2.14 ± 0.46	2.08 ± 0.44	2.02 ± 0.44	0.017	0.004
Sarcopenia (AWGS 2025), n (%)	19 (7.3)	14 (5.4)	17 (6.5)	0.673	0.721
Health status, medication and diet					
Frailty index	0.127 ± 0.072	0.136 ± 0.070	0.146 ± 0.078	0.009	0.002

Continuous variables are presented as mean ± SD and categorical variables as n (%).

P is from one-way analysis of variance or the chi-squared test across the three tertiles. P for trend is from linear or logistic regression on the tertile rank.

Tertiles are of the oral-type genera index, the mean standardised centred log-ratio abundance of Streptococcus, Veillonella, Fusobacterium, Granulicatella, Actinomyces, Rothia and Haemophilus.

ASM appendicular skeletal muscle mass, AWGS Asian Working Group for Sarcopenia, BMI body mass index, MET metabolic equivalent of task.

Characteristic	Tertile 1 (n = 261)	Tertile 2 (n = 260)	Tertile 3 (n = 261)	P	P for trend
Demographic and anthropometric					
Serum albumin, g/dL	4.20 ± 0.26	4.19 ± 0.26	4.17 ± 0.25	0.380	0.210
Number of chronic diseases	1.34 ± 1.32	1.25 ± 1.18	1.33 ± 1.22	0.612	0.916
Proton pump inhibitor use, n (%)	5 (1.9)	14 (5.4)	21 (8.0)	0.006	0.002
Laxative use, n (%)	15 (5.7)	24 (9.2)	19 (7.3)	0.315	0.504
Self-reported constipation, n (%)	97 (37.2)	95 (36.5)	78 (29.9)	0.153	0.081
Total energy intake, kcal/day	1890 ± 589	1850 ± 540	1898 ± 526	0.567	0.878
Protein, g/1000 kcal	41.8 ± 8.4	41.7 ± 9.3	39.9 ± 7.6	0.011	0.007
Dietary fibre, g/1000 kcal	7.44 ± 2.04	7.16 ± 2.42	6.91 ± 2.14	0.024	0.006
Physical activity, MET-hours/week	165.0 ± 119.9	149.3 ± 104.3	154.5 ± 120.2	0.285	0.296
Gut microbial diversity					
Observed genera	245.9 ± 51.4	215.8 ± 60.8	168.8 ± 49.4	< 0.001	< 0.001
Shannon index	6.21 ± 0.40	5.99 ± 0.54	5.59 ± 0.64	< 0.001	< 0.001
Chao1	319.2 ± 92.7	276.7 ± 96.1	209.4 ± 69.3	< 0.001	< 0.001
Continuous variables are presented as mean ± SD and categorical variables as n (%).					
P is from one-way analysis of variance or the chi-squared test across the three tertiles. P for trend is from linear or logistic regression on the tertile rank.					
Tertiles are of the oral-type genera index, the mean standardised centred log-ratio abundance of Streptococcus, Veillonella, Fusobacterium, Granulicatella, Actinomyces, Rothia and Haemophilus.					
ASM appendicular skeletal muscle mass, AWGS Asian Working Group for Sarcopenia, BMI body mass index, MET metabolic equivalent of task.					

Covariates

Covariates were chosen because they are plausible common causes of gut microbial composition and of muscle mass, and were grouped into blocks entered in a prespecified sequence rather than reported as a single fully adjusted model.

Model 1 contains age, sex and survey year. Model 2 adds body mass index. Model 3 adds total energy intake, protein intake and dietary fibre intake, all from a validated brief-type self-administered diet history questionnaire [16] and expressed per 1000 kcal for the two nutrients, together with physical activity in MET-hours per week. Model 4 adds the number of chronic diseases, current smoking, alcohol use and serum albumin. Model 5 adds the exposures that act directly on the gastrointestinal tract: proton pump inhibitor use, antibiotic use, laxative use, self-reported constipation and physician-diagnosed gastrointestinal disease. Medication use was abstracted from prescription records and classified into 16 classes.

Oral health was not available for the cohort as a whole. A dental examination was performed in 160 of the 782 participants, and in only 75 of these during the baseline survey year; the remainder were examined two to four years later. Oral variables are therefore reported as an exploratory subsample analysis and are not used for confounding adjustment.

Follow-up

Participants were invited to a second examination conducted by the same study team, at the same municipal health centres and with the same instruments and standard operating procedures as at baseline. Bioelectrical impedance, anthropometry, the frailty index, grip strength, isometric knee extension strength and the timed 10 m walk were all repeated, and were derived from the repeat measurements by the same rules as at baseline. Faecal sampling was not repeated, so the exposure is measured once, at baseline.

The interval between examinations was a mean of 3.35 years (SD 0.37, range 1.80 to 4.93). Variation in interval was handled by expressing change as the annual rate, the difference between examinations divided by each participant's own interval in years, and a sensitivity analysis added the interval as a covariate. Reasons for non-attendance were not recorded individually; the cohort does not ascertain death, and non-attendance therefore combines death, illness, relocation and declining to attend in unknown proportions, which is stated as a limitation.

Statistical analysis

The primary hypothesis, specified before analysis, was that the oral-type genera index is inversely associated with appendicular skeletal muscle mass, cross-sectionally and with its subsequent annual change. ASM is accordingly the primary outcome in both components and its P value is not corrected. The five further muscle measures, ASM/height², ASM/BMI, grip strength, knee extension strength and maximum gait speed, are secondary, and the false discovery rate across those five was controlled by the Benjamini-Hochberg procedure within each model; q values are reported alongside P values. The same procedure was applied across the five longitudinal outcomes other than the primary one, across the 130 genera within each outcome, across the six diversity-outcome pairs for each diversity measure, across the seven inflammatory markers and across the determinants of the index. Prespecification of the index

removes multiplicity across exposures but not across outcomes, and the correction is applied for that reason.

Associations were estimated by linear regression of each standardised outcome on each standardised exposure, with the covariate blocks described above. Community composition was tested by permutational multivariate analysis of variance on the Aitchison distance with 999 permutations, and dispersion was examined separately so that a shift in centroid could be distinguished from a difference in spread. Collinearity among the index and the adjustment variables was assessed by the variance inflation factor in the Model 5 design. Because the feature table was not rarefied, every model was additionally repeated with the base-10 logarithm of read depth as a covariate.

Differential attendance at the second examination was addressed in two ways. Participants with and without reassessment were compared on baseline characteristics including the index itself, and all longitudinal models were repeated with stabilised inverse probability of censoring weights derived from a logistic model of reassessment on baseline age, sex, survey year, body mass index, grip strength, frailty index, number of chronic diseases and the index.

Analyses used complete cases without imputation, in Python 3.11; two-sided P below 0.05 was considered significant. The study is reported in accordance with the STROBE guideline for observational studies. During the preparation of this work the authors used Claude (Anthropic) to assist with statistical programming and with drafting and editing the manuscript text; the authors reviewed and edited all content and take full responsibility for it.

Results

Participants

The 782 participants in the cross-sectional component had a mean age of 72.9 years, 40.3% were men, and the mean body mass index was 23.1 kg/m². Sarcopenia under AWGS 2025 was present in 6.4%. Of these participants, 493 (63.0%) attended the second examination.

Those reassessed differed from those not reassessed: they were younger (72.1 versus 74.3 years, P < 0.001), stronger (grip strength 30.2 versus 27.2 kg, P < 0.001), faster (gait speed 2.15 versus 1.95 m/s, P < 0.001) and less frail (frailty index 0.123 versus 0.159, P < 0.001). The exposure itself did not differ: the oral-type genera index was − 0.00 (SD 1.01) in those reassessed and 0.00 (SD 0.98) in those not reassessed (P = 0.959), nor did the Shannon index (P = 0.368) or body mass index (P = 0.870). Full comparisons are given in Additional file 1: Table S2. Stabilised censoring weights ranged from 0.69 to 2.63.

Characteristics across tertiles of the index

Characteristics across tertiles of the index are shown in Table 1. Age did not differ across tertiles (P for trend 0.105) and nor did body mass index (P for trend 0.795), but men became more frequent (33.3%,

39.2%, 48.3%; P for trend < 0.001), so unadjusted comparisons are not interpretable and every analysis below is adjusted for sex.

The two mass indices behaved differently. Low ASM/height² was equally common across tertiles (32.6%, 36.5%, 35.6%; P for trend 0.463) whereas low ASM/BMI became more frequent (33.3%, 39.2%, 44.4%; P for trend 0.009). Grip strength differed neither as a continuous measure (P for trend 0.182) nor as low grip strength (8.4%, 6.2%, 8.4%; P for trend 1.000), and because grip strength forms part of the AWGS definition the prevalence of sarcopenia was unchanged (7.3%, 5.4%, 6.5%; P for trend 0.721). Participants in higher tertiles were nevertheless slower, frailer, more often taking a proton pump inhibitor, lower in dietary fibre intake and markedly lower in microbial diversity.

Diversity and community composition

Read depth was correlated with observed genus richness (Spearman r = 0.31) and with Chao1 (r = 0.44) but only weakly with the Shannon index (r = 0.13), whereas the oral-type genera index, being compositional, was independent of it (r = 0.06, P = 0.090). Adjustment for read depth left every estimate in this section and the next essentially unchanged, and no conclusion depended on it. Associations of alpha diversity with muscle measures were weak and inconsistent (Table 2). Per 1 SD, observed genus richness was associated with ASM (+ 0.055 SD, 95% CI + 0.014 to + 0.096; P = 0.009, q = 0.041) and with ASM/BMI (+ 0.056, + 0.011 to + 0.100; q = 0.041), and Chao1 similarly, but no diversity measure was associated with grip strength or knee extension, and the Shannon index was associated with none of the six outcomes after correction.

Table 2
Microbial diversity and community composition in relation to muscle measures

Exposure	Muscle measure or contrast	Estimate (95% CI) or pseudo-F	P	q	n
A. Alpha diversity and muscle measures, per 1 SD of the diversity measure					
Observed genera	ASM, kg	+ 0.055 (+ 0.014 to + 0.097)	0.009	0.041	782
Observed genera	ASM/height², kg/m²	+ 0.047 (-0.001 to + 0.095)	0.055	0.110	782
Observed genera	ASM/BMI	+ 0.056 (+ 0.011 to + 0.100)	0.014	0.041	782
Observed genera	Grip strength, kg	+ 0.023 (-0.019 to + 0.066)	0.279	0.307	782
Observed genera	Knee extension strength, kg	+ 0.029 (-0.027 to + 0.086)	0.307	0.307	782
Observed genera	Maximum gait speed, m/s	+ 0.043 (-0.018 to + 0.103)	0.167	0.251	779
Shannon index	ASM, kg	+ 0.041 (-0.001 to + 0.082)	0.053	0.105	782
Shannon index	ASM/height², kg/m²	+ 0.027 (-0.020 to + 0.075)	0.258	0.348	782
Shannon index	ASM/BMI	+ 0.053 (+ 0.009 to + 0.097)	0.019	0.057	782
Shannon index	Grip strength, kg	+ 0.023 (-0.019 to + 0.065)	0.290	0.348	782
Shannon index	Knee extension strength, kg	+ 0.020 (-0.036 to + 0.076)	0.489	0.489	782
Shannon index	Maximum gait speed, m/s	+ 0.074 (+ 0.014 to + 0.134)	0.016	0.057	779

Panel A gives the difference in the standardised muscle measure per 1 SD of the diversity measure, from linear regression adjusted for age, sex and survey year. q is the Benjamini-Hochberg adjusted P value across the six muscle measures for each diversity measure. The feature table was not rarefied; repeating these models with the base-10 logarithm of read depth as a covariate left every estimate essentially unchanged.

Panel B gives permutational multivariate analysis of variance on the Aitchison distance between centred log-ratio transformed genus abundances, with 999 permutations. The pseudo-F statistic and the proportion of variance in community composition explained (R^2) are given together in the estimate column; the false discovery rate is not applied to these tests and the q column is left empty.

ASM appendicular skeletal muscle mass, BMI body mass index, CI confidence interval.

Exposure	Muscle measure or contrast	Estimate (95% CI) or pseudo-F	P	q	n
A. Alpha diversity and muscle measures, per 1 SD of the diversity measure					
Chao1	ASM, kg	+ 0.057 (+ 0.016 to + 0.099)	0.007	0.027	782
Chao1	ASM/height², kg/m²	+ 0.049 (+ 0.001 to + 0.097)	0.046	0.093	782
Chao1	ASM/BMI	+ 0.059 (+ 0.015 to + 0.104)	0.009	0.027	782
Chao1	Grip strength, kg	+ 0.031 (-0.012 to + 0.074)	0.155	0.232	782
Chao1	Knee extension strength, kg	+ 0.035 (-0.022 to + 0.092)	0.233	0.279	782
Chao1	Maximum gait speed, m/s	+ 0.033 (-0.028 to + 0.093)	0.295	0.295	779
B. Community composition, permutational analysis of variance on the Aitchison distance					
Community composition	Oral-type genera index, tertiles	pseudo-F 30.29, R^2 0.0722	0.001	—	782
Community composition	Appendicular skeletal muscle mass, tertiles	pseudo-F 1.62, R^2 0.0041	0.007	—	782
Community composition	Grip strength, tertiles	pseudo-F 1.64, R^2 0.0042	0.003	—	782
Community composition	Knee extension strength, tertiles	pseudo-F 1.85, R^2 0.0047	0.003	—	782
Community composition	Maximum gait speed, tertiles	pseudo-F 1.62, R^2 0.0042	0.011	—	779
Community composition	Sarcopenia (AWGS 2025)	pseudo-F 1.03, R^2 0.0013	0.357	—	782
Community composition	Low grip strength	pseudo-F 1.02, R^2 0.0013	0.393	—	782

Panel A gives the difference in the standardised muscle measure per 1 SD of the diversity measure, from linear regression adjusted for age, sex and survey year. q is the Benjamini-Hochberg adjusted P value across the six muscle measures for each diversity measure. The feature table was not rarefied; repeating these models with the base-10 logarithm of read depth as a covariate left every estimate essentially unchanged.

Panel B gives permutational multivariate analysis of variance on the Aitchison distance between centred log-ratio transformed genus abundances, with 999 permutations. The pseudo-F statistic and the proportion of variance in community composition explained (R^2) are given together in the estimate column; the false discovery rate is not applied to these tests and the q column is left empty.

ASM appendicular skeletal muscle mass, BMI body mass index, CI confidence interval.

Exposure	Muscle measure or contrast	Estimate (95% CI) or pseudo-F	P	q	n
A. Alpha diversity and muscle measures, per 1 SD of the diversity measure					
Community composition	Low muscle mass by either index	pseudo-F 1.64, R^2 0.0021	0.032	—	782
Panel A gives the difference in the standardised muscle measure per 1 SD of the diversity measure, from linear regression adjusted for age, sex and survey year. q is the Benjamini-Hochberg adjusted P value across the six muscle measures for each diversity measure. The feature table was not rarefied; repeating these models with the base-10 logarithm of read depth as a covariate left every estimate essentially unchanged.					
Panel B gives permutational multivariate analysis of variance on the Aitchison distance between centred log-ratio transformed genus abundances, with 999 permutations. The pseudo-F statistic and the proportion of variance in community composition explained (R^2) are given together in the estimate column; the false discovery rate is not applied to these tests and the q column is left empty.					
ASM appendicular skeletal muscle mass, BMI body mass index, CI confidence interval.					

Community composition differed across tertiles of every continuous muscle measure but by almost no diagnostic category (Table 2). Permutational analysis of variance on the Aitchison distance was significant across tertiles of grip strength (pseudo-F 1.64, R^2 0.0042, P = 0.003), knee extension strength (1.85, R^2 0.0047, P = 0.003), ASM (1.62, R^2 0.0041, P = 0.007) and gait speed (1.62, R^2 0.0042, P = 0.011). Composition did not differ by sarcopenia (P = 0.357) or by low grip strength (P = 0.393), and differed weakly by low muscle mass on either index (P = 0.032). The variance explained was small in every case, 0.4% or less.

The oral-type genera index and muscle measures

The index showed the most consistent associations of the four exposures (Table 3). In Model 1, each 1 SD of the index was associated with lower ASM (-0.075 SD, 95% CI -0.116 to -0.034; P < 0.001), ASM/height² (-0.058, -0.105 to -0.010), ASM/BMI (-0.054, -0.098 to -0.010), knee extension strength (-0.074, -0.130 to -0.017) and maximum gait speed (-0.093, -0.153 to -0.033). Grip strength was not associated (-0.038, -0.081 to + 0.004).

Table 3
The oral-type genera index and muscle measures under five prespecified adjustment models

Model	Covariates	Estimate (95% CI)	P	q	n
ASM, kg (primary outcome)					
Model 1	Age, sex, survey year	-0.075 (-0.116 to -0.034)	< 0.001		782
Model 2	Model 1 plus body mass index	-0.069 (-0.106 to -0.032)	< 0.001		782
Model 3	Model 2 plus energy, protein and fibre intake and physical activity	-0.064 (-0.101 to -0.026)	< 0.001		780
Model 4	Model 3 plus comorbidity count, smoking, alcohol and albumin	-0.064 (-0.102 to -0.026)	< 0.001		775
Model 5	Model 4 plus proton pump inhibitor, antibiotic and laxative use, constipation and gastrointestinal disease	-0.067 (-0.105 to -0.029)	< 0.001		775
ASM/height², kg/m²					
Model 1	Age, sex, survey year	-0.058 (-0.105 to -0.010)	0.018	0.022	782
Model 2	Model 1 plus body mass index	-0.046 (-0.081 to -0.012)	0.009	0.015	782
Model 3	Model 2 plus energy, protein and fibre intake and physical activity	-0.041 (-0.075 to -0.007)	0.020	0.024	780
Model 4	Model 3 plus comorbidity count, smoking, alcohol and albumin	-0.042 (-0.077 to -0.008)	0.016	0.027	775
Model 5	Model 4 plus proton pump inhibitor, antibiotic and laxative use, constipation and gastrointestinal disease	-0.046 (-0.081 to -0.012)	0.009	0.015	775
ASM/BMI					
Model 1	Age, sex, survey year	-0.054 (-0.098 to -0.010)	0.016	0.022	782
Model 2	Model 1 plus body mass index	-0.062 (-0.100 to -0.023)	0.002	0.005	782
Model 3	Model 2 plus energy, protein and fibre intake and physical activity	-0.057 (-0.095 to -0.018)	0.004	0.016	780
Model 4	Model 3 plus comorbidity count, smoking, alcohol and albumin	-0.057 (-0.096 to -0.018)	0.004	0.020	775

Model	Covariates	Estimate (95% CI)	P	q	n
ASM, kg (primary outcome)					
Model 5	Model 4 plus proton pump inhibitor, antibiotic and laxative use, constipation and gastrointestinal disease	-0.059 (-0.098 to -0.020)	0.003	0.011	775
Grip strength, kg					
Model 1	Age, sex, survey year	-0.038 (-0.081 to + 0.004)	0.077	0.077	782
Model 2	Model 1 plus body mass index	-0.038 (-0.080 to + 0.004)	0.079	0.079	782
Model 3	Model 2 plus energy, protein and fibre intake and physical activity	-0.035 (-0.077 to + 0.008)	0.111	0.111	780
Model 4	Model 3 plus comorbidity count, smoking, alcohol and albumin	-0.029 (-0.071 to + 0.014)	0.188	0.188	775
Model 5	Model 4 plus proton pump inhibitor, antibiotic and laxative use, constipation and gastrointestinal disease	-0.036 (-0.079 to + 0.007)	0.102	0.102	775
Knee extension strength, kg					
Model 1	Age, sex, survey year	-0.074 (-0.130 to -0.017)	0.011	0.022	782
Model 2	Model 1 plus body mass index	-0.070 (-0.125 to -0.014)	0.014	0.017	782
Model 3	Model 2 plus energy, protein and fibre intake and physical activity	-0.068 (-0.124 to -0.013)	0.016	0.024	780
Model 4	Model 3 plus comorbidity count, smoking, alcohol and albumin	-0.063 (-0.119 to -0.007)	0.026	0.033	775
Model 5	Model 4 plus proton pump inhibitor, antibiotic and laxative use, constipation and gastrointestinal disease	-0.067 (-0.123 to -0.010)	0.021	0.026	775
Maximum gait speed, m/s					
Model 1	Age, sex, survey year	-0.093 (-0.153 to -0.033)	0.003	0.013	779
Model 2	Model 1 plus body mass index	-0.095 (-0.155 to -0.035)	0.002	0.005	779
Model 3	Model 2 plus energy, protein and fibre intake and physical activity	-0.084 (-0.144 to -0.024)	0.006	0.016	777
Model 4	Model 3 plus comorbidity count, smoking, alcohol and albumin	-0.080 (-0.140 to -0.020)	0.009	0.022	772

Model	Covariates	Estimate (95% CI)	P	q	n
ASM, kg (primary outcome)					
Model 5	Model 4 plus proton pump inhibitor, antibiotic and laxative use, constipation and gastrointestinal disease	-0.088 (-0.148 to -0.027)	0.004	0.011	772
Estimates are the difference in the standardised muscle measure per 1 SD of the index, so that −0.067 means that a 1 SD higher index corresponds to a 0.067 SD lower outcome.					
Model 1: age, sex, survey year. Model 2: Model 1 plus body mass index. Model 3: Model 2 plus total energy intake, protein intake per 1000 kcal, dietary fibre intake per 1000 kcal and physical activity in MET-hours per week. Model 4: Model 3 plus number of chronic diseases, current smoking, alcohol use and serum albumin. Model 5: Model 4 plus proton pump inhibitor use, antibiotic use, laxative use, self-reported constipation and gastrointestinal disease.					
ASM is the primary outcome and its P value is not corrected. q is the Benjamini-Hochberg adjusted P value across the five secondary outcomes within each model. No variance inflation factor in the Model 5 design exceeded 2.17.					
ASM appendicular skeletal muscle mass, BMI body mass index, CI confidence interval.					

The associations were not explained by the covariates. In Model 5, which adds body mass index, energy, protein and fibre intake, physical activity, comorbidity, smoking, alcohol, albumin, proton pump inhibitor use, antibiotic use, laxative use, constipation and gastrointestinal disease, the estimate for the primary outcome was − 0.067 SD (-0.105 to -0.029; P < 0.001; n = 775). Among the secondary outcomes, ASM/BMI (-0.059, -0.098 to -0.020; q = 0.011), maximum gait speed (-0.088, -0.148 to -0.027; q = 0.011), ASM/height² (-0.046, -0.081 to -0.012; q = 0.015) and knee extension strength (-0.067, -0.123 to -0.010; q = 0.026) all remained significant after control of the false discovery rate, whereas grip strength did not (-0.036, -0.079 to + 0.007; q = 0.102). No variance inflation factor in this model exceeded 2.17, so collinearity among the index and the adjustment variables was not material. Adding read depth to Model 5 changed the primary estimate by less than 0.001 SD (-0.067, -0.105 to -0.029).

Associations were graded across tertiles of the index (Fig. 1). Adjusted for age, sex and survey year, the third tertile differed from the first by -0.143 SD in ASM (P for trend 0.006), -0.220 SD in gait speed (P for trend 0.003) and − 0.144 SD in knee extension strength (P for trend 0.040).

The association with knee extension strength reflected muscle quantity rather than force per unit muscle: it was attenuated when force was expressed per body weight and abolished when expressed per leg lean mass. Omitting any single constituent genus changed the primary estimate by at most 0.004 SD, so the index was not driven by any one organism (Additional file 1: Table S3).

Individual genera

Of the 130 genera tested against each outcome, few survived correction (Table 4). Bifidobacterium was associated with ASM (-0.082; q = 0.013), Anaerotruncus with ASM/height² (-0.100; q = 0.006) and

Megasphaera with grip strength (-0.079; q = 0.030). No genus survived correction for ASM/BMI, knee extension strength or gait speed, although 14 to 20 genera reached nominal significance for each outcome. Two constituents of the index were among the strongest signals for gait speed, Streptococcus (-0.091) and, at the same q value, Lactobacillus (-0.102), neither surviving correction (q = 0.098).

Table 4
The five genera most strongly associated with each muscle measure, of 130 tested

Muscle measure	Genus	Estimate	P	q
ASM, kg (genera reaching nominal significance: 18 of 130)				
	Bifidobacterium	-0.082	< 0.001	0.013
	Anaerotruncus	-0.068	0.001	0.060
	Haemophilus	-0.068	0.001	0.060
	Enterococcus	-0.062	0.003	0.088
	Marvinbryantia	+ 0.062	0.003	0.088
ASM/height², kg/m² (genera reaching nominal significance: 21 of 130)				
	Anaerotruncus	-0.100	< 0.001	0.006
	Marvinbryantia	+ 0.075	0.002	0.117
	Enterococcus	-0.069	0.005	0.209
	Dorea	+ 0.064	0.009	0.225
	Bifidobacterium	-0.059	0.015	0.225
ASM/BMI (genera reaching nominal significance: 14 of 130)				
	Bacteroides	-0.058	0.010	0.244
	Lactobacillus	-0.065	0.005	0.244
	Bifidobacterium	-0.061	0.007	0.244
	Fusobacterium	-0.059	0.008	0.244
	Oscillospira	+ 0.057	0.011	0.244
Grip strength, kg (genera reaching nominal significance: 16 of 130)				
	Megasphaera	-0.079	< 0.001	0.030
	Bifidobacterium	-0.067	0.002	0.118
	Coprococcus	+ 0.064	0.003	0.118

Estimates are the difference in the standardised muscle measure per 1 SD of the standardised centred log-ratio abundance of the genus, adjusted for age, sex and survey year (n = 772 to 782).

q is the Benjamini-Hochberg adjusted P value across all 130 genera within each muscle measure. Genera are ordered by q within each muscle measure.

ASM appendicular skeletal muscle mass, BMI body mass index.

Muscle measure	Genus	Estimate	P	q
ASM, kg (genera reaching nominal significance: 18 of 130)				
	Enterococcus	-0.060	0.005	0.177
	Roseburia	+ 0.055	0.009	0.224
Knee extension strength, kg (genera reaching nominal significance: 18 of 130)				
	[Ruminococcus]_gauvreauii_group	+ 0.094	< 0.001	0.121
	Actinomyces	-0.083	0.004	0.169
	Moryella	+ 0.082	0.004	0.169
	Coprobacillus	-0.079	0.006	0.197
	Intestinibacter	-0.076	0.008	0.202
Maximum gait speed, m/s (genera reaching nominal significance: 20 of 130)				
	Streptococcus	-0.090	0.003	0.098
	Lactobacillus	-0.102	< 0.001	0.098
	Lachnospiraceae_UCG-010	+ 0.095	0.002	0.098
	Rothia	-0.094	0.003	0.098
	Clostridium_sensu_stricto_1	-0.079	0.010	0.207
Estimates are the difference in the standardised muscle measure per 1 SD of the standardised centred log-ratio abundance of the genus, adjusted for age, sex and survey year (n = 772 to 782).				
q is the Benjamini-Hochberg adjusted P value across all 130 genera within each muscle measure. Genera are ordered by q within each muscle measure.				
ASM appendicular skeletal muscle mass, BMI body mass index.				

Three-year change in muscle mass

Among the 493 participants reassessed, no diversity measure was associated with annual change in ASM (observed richness + 0.062%/year, P = 0.261; Shannon + 0.053, P = 0.311; Chao1 + 0.067, P = 0.221), and no genus survived correction for any longitudinal outcome.

The index was associated with subsequent loss of muscle mass (Table 5). Per 1 SD, annual change in ASM was − 0.134%/year (95% CI -0.242 to -0.027; P = 0.014) adjusted for age, sex, survey year and baseline ASM, and − 0.125%/year (-0.235 to -0.015; P = 0.025; n = 488) in the fully adjusted Model 5. Annual change in ASM/height² was − 0.0066 kg/m² per year (-0.0131 to -0.0000; P = 0.049), although this did not survive correction across the five secondary longitudinal outcomes (q = 0.148). Weighting for differential attendance strengthened rather than attenuated these estimates: with censoring weights,

annual change in ASM was − 0.180%/year (-0.292 to -0.067; P = 0.002) and in ASM/height² -0.0093 kg/m² per year (-0.0159 to -0.0026; P = 0.006). Adding the examination interval, or read depth, as a covariate did not change the estimates (with read depth, -0.126%/year, -0.236 to -0.016).

Table 5
The oral-type genera index and 3-year change in muscle mass, strength and physical performance

Model	Covariates	Estimate (95% CI)	P	q	n
Annual change in ASM, %/year (primary longitudinal outcome)					
Model 1	Age, sex, survey year, baseline value of the outcome	-0.134 (-0.242 to -0.027)	0.014		493
Model 5	Model 1 plus the full covariate set of Table 3, Model 5	-0.125 (-0.235 to -0.015)	0.025		488
Model 5 weighted	Model 5 with inverse probability of censoring weights	-0.180 (-0.292 to -0.067)	0.002		488
Annual change in ASM, kg/year					
Model 1	Age, sex, survey year, baseline value of the outcome	-0.0175 (-0.0354 to + 0.0004)	0.055		493
Model 5	Model 1 plus the full covariate set of Table 3, Model 5	-0.0176 (-0.0358 to + 0.0007)	0.059	0.148	488
Model 5 weighted	Model 5 with inverse probability of censoring weights	-0.0256 (-0.0440 to -0.0073)	0.006		488
Annual change in ASM/height², kg/m² per year					
Model 1	Age, sex, survey year, baseline value of the outcome	-0.0066 (-0.0131 to -0.0000)	0.048		493
Model 5	Model 1 plus the full covariate set of Table 3, Model 5	-0.0066 (-0.0131 to -0.0000)	0.049	0.148	488
Model 5 weighted	Model 5 with inverse probability of censoring weights	-0.0093 (-0.0159 to -0.0026)	0.006		488
Annual change in grip strength, kg/year					
Model 1	Age, sex, survey year, baseline value of the outcome	-0.001 (-0.082 to + 0.080)	0.986		493
Model 5	Model 1 plus the full covariate set of Table 3, Model 5	-0.019 (-0.100 to + 0.061)	0.638	0.798	488

Model	Covariates	Estimate (95% CI)	P	q	n
Annual change in ASM, %/year (primary longitudinal outcome)					
Model 5 weighted	Model 5 with inverse probability of censoring weights	-0.056 (-0.137 to + 0.025)	0.177		488
Annual change in knee extension strength, kg/year					
Model 1	Age, sex, survey year, baseline value of the outcome	-0.123 (-0.282 to + 0.037)	0.132		489
Model 5	Model 1 plus the full covariate set of Table 3, Model 5	-0.104 (-0.269 to + 0.060)	0.214	0.357	484
Model 5 weighted	Model 5 with inverse probability of censoring weights	-0.113 (-0.275 to + 0.050)	0.173		484
Annual change in maximum gait speed, m/s per year					
Model 1	Age, sex, survey year, baseline value of the outcome	-0.00303 (-0.01090 to + 0.00483)	0.449		490
Model 5	Model 1 plus the full covariate set of Table 3, Model 5	-0.00061 (-0.00853 to + 0.00732)	0.881	0.881	485
Model 5 weighted	Model 5 with inverse probability of censoring weights	-0.00081 (-0.00858 to + 0.00695)	0.837		485
Estimates are the difference in annual change per 1 SD of the index, among the 493 participants reassessed a mean of 3.35 years after baseline (SD 0.37, range 1.80 to 4.93). Change is expressed as the difference between examinations divided by each participant's own interval in years. Knee extension strength and gait speed were available at both examinations in 489 and 490 participants respectively.					
Model 1 is adjusted for age, sex, survey year and the baseline value of the outcome. Model 5 adds the full covariate set listed in the legend to Table 3.					
The weighted models use stabilised inverse probability of censoring weights from a logistic model of reassessment on baseline age, sex, survey year, body mass index, grip strength, frailty index, number of chronic diseases and the index (weight range 0.69 to 2.63).					
Annual change in ASM expressed as a percentage is the primary longitudinal outcome and its P value is not corrected; q is the Benjamini-Hochberg adjusted P value across the other five longitudinal outcomes.					
ASM appendicular skeletal muscle mass, CI confidence interval.					

The index was not associated with change in any measure of strength or performance. Change in grip strength was unrelated to it in every model (Model 1, -0.001 kg/year, P = 0.986; Model 5, -0.019 kg/year,

-0.100 to + 0.061, P = 0.638; q = 0.798). Knee extension strength fell by a mean of 0.13 kg/year over the interval (29.0 to 28.6 kg, n = 489) and maximum gait speed by 0.041 m/s per year (2.15 to 2.02 m/s, n = 490), but neither change was associated with the index: knee extension − 0.104 kg/year per 1 SD (-0.269 to + 0.060; P = 0.214; q = 0.357) and gait speed − 0.0006 m/s per year (-0.0085 to + 0.0073; P = 0.881; q = 0.881) in Model 5, with censoring weights giving − 0.113 and − 0.0008 respectively. The two measures that showed the clearest cross-sectional associations with the index therefore showed none prospectively.

Determinants of the index, inflammation and oral findings

Three determinants of the index were identified among 15 candidates after control of the false discovery rate (Table 6): proton pump inhibitor use (+ 0.492 SD, 95% CI + 0.176 to + 0.808; q = 0.017), male sex (+ 0.229, + 0.086 to + 0.371; q = 0.017) and dietary fibre intake (-0.100 SD per 1 SD, -0.173 to -0.027; q = 0.038). Proton pump inhibitor use and fibre intake were mutually independent (+ 0.495 and − 0.101 respectively when entered together). Self-reported constipation was associated with a lower index (-0.185, -0.332 to -0.038; q = 0.051). Antibiotic use, laxative use, gastrointestinal disease, smoking and alcohol were not associated.

Table 6
Determinants of the oral-type genera index among 15 prespecified candidates

Candidate determinant	n or users	Estimate (95% CI)	P	q
Dietary fibre, per 1 SD	782	-0.100 (-0.173 to -0.027)	0.008	0.038
Protein, per 1 SD	782	-0.081 (-0.154 to -0.008)	0.029	0.086
Total energy intake, per 1 SD	782	-0.055 (-0.128 to + 0.018)	0.140	0.349
Physical activity, per 1 SD	780	+ 0.023 (-0.052 to + 0.097)	0.548	0.747
Age, per 1 SD	782	+ 0.045 (-0.028 to + 0.117)	0.227	0.426
Body mass index, per 1 SD	782	-0.024 (-0.094 to + 0.046)	0.495	0.742
Male sex	315	+ 0.229 (+ 0.086 to + 0.371)	0.002	0.017
Proton pump inhibitor use	40	+ 0.492 (+ 0.176 to + 0.808)	0.002	0.017
Laxative use	58	+ 0.032 (-0.241 to + 0.304)	0.819	0.942
Antibiotic use	5	+ 0.172 (-0.703 to + 1.048)	0.700	0.875
NSAID use	20	+ 0.157 (-0.283 to + 0.598)	0.483	0.742
Self-reported constipation	270	-0.185 (-0.332 to -0.038)	0.014	0.051
Gastrointestinal disease	147	+ 0.013 (-0.173 to + 0.199)	0.890	0.942
Current smoking	203	-0.008 (-0.219 to + 0.204)	0.942	0.942
Alcohol use	307	-0.110 (-0.271 to + 0.051)	0.180	0.387
Estimates are the difference in the index, in SD units, per 1 SD of the continuous candidate or for the presence of the binary candidate, adjusted for age, sex and survey year.				
For binary candidates the second column gives the number of participants with the characteristic; for continuous candidates it gives the number analysed.				
q is the Benjamini-Hochberg adjusted P value across the 15 candidates. Dietary fibre and proton pump inhibitor use remained associated when entered together (-0.101 and + 0.495 respectively).				
CI confidence interval, NSAID non-steroidal anti-inflammatory drug.				

None of seven circulating inflammatory or oxidative markers was associated with the index after correction (Additional file 1: Table S4), the closest being immunoglobulin A (+ 0.062 SD per 1 SD, -0.008

to + 0.133; q = 0.331) and interleukin-6 (+ 0.053, -0.018 to + 0.124; q = 0.331). Adjustment for interleukin-6 or C-reactive protein left the association between the index and ASM unchanged.

In the 160 participants with a dental examination, of whom only 75 were examined during the baseline survey year, the index was not associated with any oral measure (Additional file 1: Table S5): masticatory ability (Spearman r = -0.02, P = 0.78), the Community Periodontal Index (r = -0.09, P = 0.26), oral bacterial count (r = -0.07, P = 0.59), tongue coating (r = + 0.14, P = 0.33) or the EAT-10 swallowing score (r = + 0.24, P = 0.08). These analyses are underpowered and the timing of the dental examination does not permit a causal reading, so they neither support nor exclude an oral origin for the index genera.

Sex-stratified estimates

Estimates were examined by sex because a previous Japanese study reported sex-specific associations [17] (Additional file 1: Table S6). Associations with muscle mass were larger in men (ASM − 0.129, P = 0.007; ASM/height² -0.096, P = 0.013) than in women (-0.077, P = 0.059; -0.036, P = 0.299), whereas the association with gait speed was confined to women (-0.127, P = 0.002; men − 0.044, P = 0.373), as was the longitudinal association with muscle loss (women − 0.187%/year, P = 0.013; men − 0.097, P = 0.243). No interaction with sex approached significance (all P for interaction 0.14 or above), so these differences are not established and the pooled estimates are preferred.

Discussion

In 782 community-dwelling older adults, an index of oral-type gut genera specified in advance on biological grounds was inversely associated with appendicular muscle mass by both AWGS 2025 indices, with knee extension strength and with gait speed, and in the 493 reassessed it was associated with subsequent loss of muscle mass. The associations were small, between 0.05 and 0.09 SD per 1 SD of the index, and they persisted through a covariate sequence that ended with gastric acid suppression, antibiotics, laxatives, constipation and gastrointestinal disease. Diversity showed weak and inconsistent cross-sectional associations and none with subsequent change.

Diversity may be the wrong resolution

Much of the human gut-muscle literature is organised around diversity, and our data suggest that this framing loses the signal. No diversity measure was associated with 3-year change, and cross-sectional associations were inconsistent, whereas in the same participants a seven-genus index defined in advance was associated with five of six cross-sectional outcomes, was graded across tertiles and was associated with longitudinal change. Had we reported diversity alone we would have concluded that the microbiome was unrelated to muscle health.

The same contrast appeared for composition, which differed across tertiles of every continuous muscle measure but not by sarcopenia or by low grip strength. Dichotomisation discarded the signal that the continuous measure retained. We would emphasise, however, that the variance in composition explained

by any muscle measure was 0.4% or less, so these are statements about detectability rather than about magnitude.

Comparison with previous human studies

Reviews report no difference in alpha diversity between frail sarcopenic older adults and healthy controls despite differences in individual taxa [1, 2]. Our data reproduce that dissociation with repeated measurement: diversity showed no association with 3-year change while a taxonomic index did. The most comparable study, of 356 Japanese outpatients applying the AWGS 2019 criteria, found associations confined to men [17]. We partly reproduce this, in that associations with muscle mass were larger in men, but associations with gait speed and with longitudinal change were larger in women and no interaction was significant, so we do not regard the sex difference as established.

Quantity rather than quality, and the case for lower-limb measures

Two patterns bear on how muscle function should be measured. First, the index was associated with knee extension strength but not with grip strength, and composition differed more strongly across knee extension tertiles than across grip tertiles. Although grip strength is the AWGS strength criterion, it assesses a small distal muscle group. Had we measured grip alone we would have reported no association with strength, and none with sarcopenia, since grip strength forms part of that diagnosis.

Second, normalisation abolished the association with knee extension strength: the index was unrelated to force per body weight or per leg lean mass. The index therefore tracks muscle quantity rather than force-generating capacity. The longitudinal data support that reading directly: the index was associated with subsequent change in muscle mass but not with change in knee extension strength or gait speed, even though those were the measures most strongly associated with it cross-sectionally. This constrains any mechanism to one acting on muscle protein balance rather than on neuromuscular activation or contractile quality, and it is a caution against reading the cross-sectional strength and performance associations as evidence of a process that is still unfolding.

Mechanism, and what we could not test

Faecal and subgingival communities are more similar in older than in younger adults, and several oral taxa are detected more often in the faeces of older people [18]. Swallowed oral pathobionts can colonise the gut and disrupt the intestinal barrier [19]; in mice, Porphyromonas gingivalis reduces ileal tight-junction gene expression and produces systemic inflammation and insulin resistance [20]. Ectopic colonisation induces T-helper-1 responses [8], and age-related permeability permits lipopolysaccharide to enter the circulation, promoting the inflammatory signalling of the ageing muscle phenotype [1, 2].

Our data support one step of this account and not another. The step they support is the gastric acid barrier: proton pump inhibitor users had an index almost half a standard deviation higher, which replicates established findings [6, 7] and shows that the index responds to a known perturbation of that

barrier. The step they do not support is systemic inflammation, since no circulating marker was associated with the index and adjustment for interleukin-6 or C-reactive protein left the muscle association unchanged. A local intestinal action, a microbial metabolite not measured here, or reverse causation would fit our data better than a systemic inflammatory pathway. Short-chain fatty acids were not measured.

The dental substudy, which we had hoped would speak to oral origin, does not. With 160 participants examined, only 75 of them concurrently with the baseline survey, and no association with any oral measure, it is uninformative rather than negative. The name we give the index is therefore descriptive, and we do not claim that the faecal sequences originated in the mouth of the individual concerned.

Direction of effect and residual confounding

Baseline muscle mass was itself associated with the index, so the cross-sectional component cannot establish direction. The longitudinal component is more informative, in that the exposure precedes the change and the association persists after adjustment for the baseline value of the outcome, but it does not establish direction either. Subclinical illness that alters gastrointestinal physiology while accelerating muscle loss would produce the same pattern, and because the cohort does not ascertain death or hospitalisation we cannot examine that possibility directly. Residual confounding is likely despite the covariate sequence: diet was self-reported, physical activity was self-reported, oral health was unavailable for 80% of the cohort, and medication was recorded as current use rather than as cumulative exposure.

Strengths and limitations

The strengths are a population-based sample, an index specified in advance on biological grounds rather than selected from the data, repeat measurement of muscle mass, strength and gait speed over three years, both AWGS 2025 mass indices, three functional measures including lower-limb strength, an explicit covariate sequence, control of the false discovery rate across outcomes, and complete reporting of null results.

The limitations are substantial. The effect sizes are small, between 0.05 and 0.09 SD per 1 SD, which is unlikely to be clinically meaningful in an individual and is better read as evidence that a relationship exists than as a basis for prediction. Stool was collected once, so within-person variability in the exposure is not captured and the association is attenuated to an unknown degree. Profiling at genus level by 16S does not resolve species or function. Attendance at the second examination was 63% and those reassessed were younger, stronger and less frail; the exposure itself did not differ between the two groups, and censoring weights strengthened rather than weakened the longitudinal estimates, but unmeasured reasons for non-attendance, including death, cannot be excluded. The prospective findings rest on a single repeat measurement after a mean of 3.35 years, which gives no information about the shape of the trajectory, and the associations with change were confined to muscle mass; strength and performance showed none. Sarcopenia was present in only 6.4% of participants, so conclusions rest on

continuous measures. Participants came from a single rural region of Japan with a distinctive dietary pattern and body size distribution.

Conclusions

In this population-based cohort of older Japanese adults, an index of oral-type gut genera was inversely associated with appendicular muscle mass, knee extension strength and gait speed, and with subsequent 3-year loss of muscle mass though not with change in strength or gait speed, independently of nutrition, physical activity, comorbidity and gastrointestinal medication, whereas microbial diversity was associated with none of these. The associations are small and the design is observational, so they identify a relationship rather than establish causation or support prediction in an individual. The findings suggest that the gut-muscle axis is better studied at taxonomic resolution than through diversity, against continuous muscle measures rather than the AWGS diagnostic category, and with lower-limb strength and gait speed rather than grip strength alone.

Abbreviations

ASM
Appendicular skeletal muscle mass
ASV
Amplicon sequence variant
AWGS
Asian Working Group for Sarcopenia
BMI
Body mass index
CI
Confidence interval
CLR
Centred log-ratio
EAT-10
Eating Assessment Tool-10
MET
Metabolic equivalent of task
PCR
Polymerase chain reaction
PPI
Proton pump inhibitor
SD
Standard deviation
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine (approval number ERB-C-885-10) and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The raw 16S rRNA gene sequencing data generated in this study have been deposited in the National Center for Biotechnology Information Sequence Read Archive under accession numbers PRJNA1470357 and PRJNA1440982, and the corresponding subject-level identifier list (Microbiota-ID, age, sex and body mass index) is provided in Additional file 1: Table S1. The participant-level clinical data are not publicly available because they contain information that could compromise the privacy of participants in a small rural community, and because the consent obtained does not cover open deposition; they are available from the corresponding author on reasonable request and with the permission of the Ethics Committee of Kyoto Prefectural University of Medicine.

Competing interests

YN has received scholarship funds from Taiyo Kagaku Co. Ltd., Morinaga Co. Ltd., Miyarisan Pharmaceutical Co. Ltd., Morishita-Jintan Co. Ltd., Fujikko Co. Ltd., Mizkan Co. Ltd., Mykinso Co. Ltd. and KINS Co. Ltd.; collaborative research funds from TOTO Ltd., Takeda Pharmaceutical Co. Ltd. and Taiyo Kagaku Co. Ltd.; and lecture fees from Takeda Pharmaceutical Co. Ltd., Biofermin Co. Ltd. and Miyarisan Pharmaceutical Co. Ltd. TT has received a collaborative research fund from PreMedica Inc. and lecture fees from Mochida Pharmaceutical Co. Ltd., Janssen Pharmaceutical K.K., EA Pharma Co. Ltd., Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical Co. Ltd., AbbVie GK, Bristol-Myers Squibb and Pfizer Japan Inc. The other authors declare that they have no competing interests.

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Authors' contributions

YN conceived the study, drafted the manuscript and obtained funding. HK and KM performed the statistical analysis and validated the results. NO, AA and TKa carried out the field examinations and project administration. TY performed the dental examinations. JN, TKi and SM contributed to the field examinations and project administration, and SM obtained funding. RI designed and performed the microbiome analysis. TT conceived the study, obtained funding and revised the manuscript. All authors read and approved the final manuscript.

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Figures

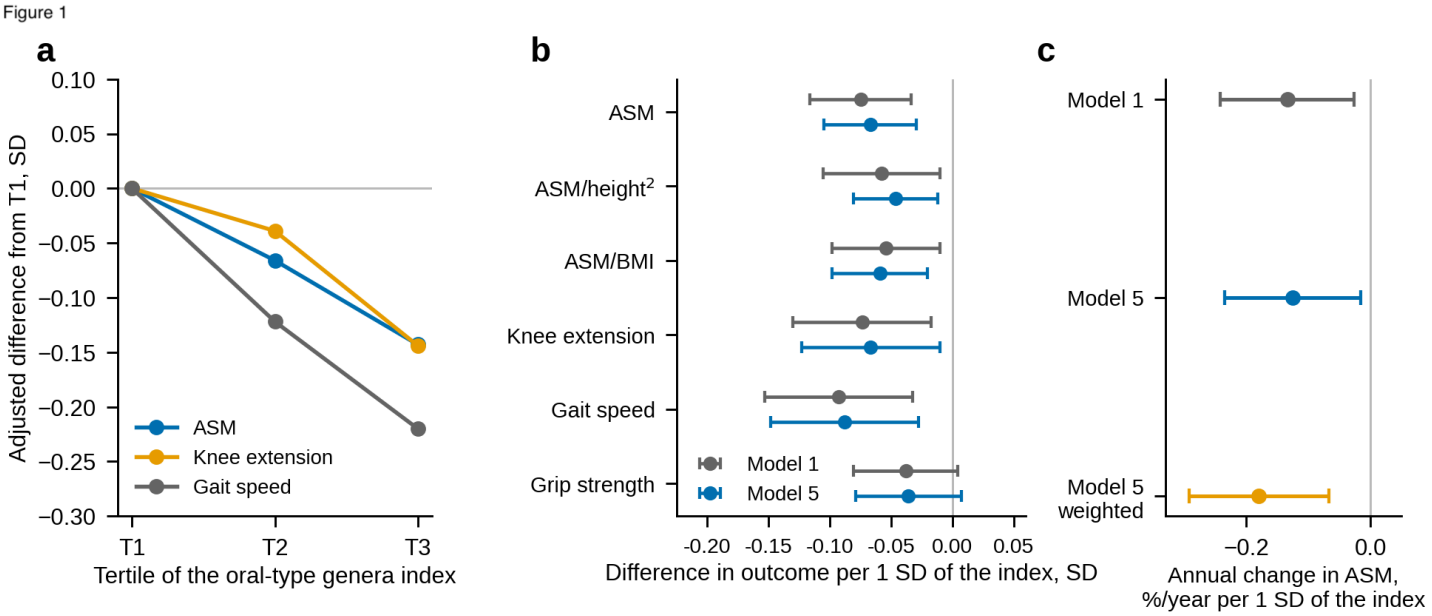


Figure 1

Muscle measures across tertiles of the oral-type genera index, and the association of the index with 3-year change in appendicular skeletal muscle mass. a Adjusted mean difference from the first tertile, in SD units, for appendicular skeletal muscle mass, knee extension strength and maximum gait speed, with 95% confidence intervals; models are adjusted for age, sex and survey year (n = 782). b Adjusted difference in each muscle measure per 1 SD of the index, in SD units, under Model 1 and Model 5 (n = 775 to 782). c Adjusted annual change in appendicular skeletal muscle mass per 1 SD of the index, unweighted and with inverse probability of censoring weights (n = 488). Error bars are 95% confidence intervals throughout.

Supplementary Files

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- TableS1.pdf
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