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Almeida, Helena Maia; Veiga Sardeli, Amanda; Conway, Jessica; Duggal, Niharika; Cavaglieri, Cláudia Regina

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Comparison between frail and non-frail older adults' gut microbiota: A systematic review and meta-analysis.

Abstract

Background

Emerging evidence suggests that the intestinal microbiota (IM) undergoes remodelling as we age, and this impacts the ageing trajectory and mortality in older adults. The aim was to investigate IM diversity differences between frail and non-frail older adults by meta-analysing previous studies.

Methods

The protocol of this systematic review with meta-analysis was registered on PROSPERO (CRD42021276733). We searched for studies comparing IM diversity of frail and non-frail older adults, indexed on PubMed, Embase, Cochrane and Web of Science, in November, 2021.

Results

We included 11 studies with 1239 participants, of which 340 were meta-analysed. Frailty was defined by a variety of criteria (i.e. Fried Scale, European Consensus on Sarcopenia). There were no differences in the meta-analyses between the frail and non-frail groups for species richness index (SMD=-0.147; 95% CI -0.394; 0.100, p=0.243) and species diversity index (SMD=-0.033; 95% IC -0.315; 0.250; p=0.820). However, we identified almost 50 differences between frail and non-frail within the relative abundance of bacteria phyla, families, genera, and species in the primary studies.

Conclusions

The evidence to prove that there are differences between frail and non-frail IM diversity by meta-analysis is still lacking. The present results suggest that further investigation into role of specific bacteria, their function, and their influence on the physiopathology of frailty is needed.

Keywords: Frailty; Sarcopenia; Gut microbiome; Microbiota, Ageing.

1. Introduction

Although advances in public health and medical care have led to a global expansion of the population aged 65 years and above, the biggest problem is that healthy life expectancy has not kept pace with lifespan extension (House of Lords, 2021). Thus, understanding the potential contributors towards the ageing process is fundamental to bridge the gap between lifespan and health span (Campisi et al., 2019; Kapahi et al., 2010). Frailty is a multifactor geriatric syndrome that is characterized by an increased vulnerability to adverse health outcomes, which include defects in physical functioning, diminished muscle strength, exhaustion and unintentional weight loss, culminating in a reduced quality of life, loss of independence, and increased risk of hospitalisation and mortality in older adults (Fried et al., 2001; Hoogendijk et al., 2019). As a result, it is unsurprising that screening for frailty in older adults using the Fried (Fried et al., 2001) and Rockwood (Rockwood et al., 2005) frailty tools is becoming routine clinical practice (National Health Service, 2019). Moreover, the pathophysiology of frailty is complex and incorporates multiple interconnected pathways that are poorly understood.

In the past decade, we have seen a growing interest in the intestinal microbiome (IM) for its role in regulating multiple aspects of health, including nutrient absorption, carbohydrate fermentation, immune system regulation, central nervous system development, and skeletal muscle metabolism (Bäckhed et al., 2004; Saint-Georges-Chaumet and Edeas, 2016; Sovran et al., 2019; van de Wouw et al., 2017). The human IM remains relatively stable throughout adult life, but the microbiome composition undergoes compositional and functional changes with advancing age. This results in reduced IM biodiversity and a state of microbial dysbiosis (Maffei et al., 2017) that is accompanied by a loss of core commensal bacterial species (Bacteriodetes, Bifidobacterium) and an expansion of opportunistic microbes (Fusobacterium, E.coli) (Santoro et al., 2018). Microbial dysbiosis with ageing has been associated with a range of age-related conditions, including dementia, autoimmune inflammatory diseases, osteoporosis, increased risk of cardiovascular disease, and physical frailty (Brunt et al., 2019; Calvani et al., 2018; Karlsson et al., 2012; Ohlsson and Sjögren, 2015; Picca and Calvani, 2020). Furthermore, microbial compositional changes are accompanied by a shift in the microbial metabolite profile, namely a decline in short chain fatty acids (SCFAs) which play a key role in regulating host physiological processes (Conway and A Duggal, 2021; Rampelli et al., 2013).

The IM has been shown to impact skeletal muscle metabolism; thus, it makes sense that multiple studies have reported an association between microbial dysbiosis and the loss of muscle mass and function (Poggiogalle et al., 2019; Ticinesi et al., 2019). Claesson *et al.*, showed that older adults living in a long-term care centre had a significantly less diverse IM than residents within the community, and the reduction in community-associated biodiversity was correlated with an increase in frailty (Claesson *et al.*, 2012). On the other hand, a study by Milani *et al.*, conducted with hospitalized older adults showed a low association between IM diversity and the presence of frailty (Milani et al., 2016). It highlights an urgent need of understanding the underlying pathophysiology driving frailty to elucidate some targetable mechanisms.

Furthermore, age-associated microbial dysbiosis has also been associated with increased intestinal membrane barrier permeability (Tran and Greenwood-Van Meerveld, 2013), resulting in translocation of microbial products and toxins into circulation, which has recently been identified as a contributing factor towards the age-associated increase in basal inflammation in aged individuals, termed inflammageing (Thevaranjan et al., 2017). Pro-inflammatory cytokines have been reported to induce muscle degradation via the ubiquitin proteasome pathway and have been recognised as potential contributors towards frailty (Soysal et al., 2016). Thus, it is safe to hypothesise an involvement of the gut microbiota in driving frailty in older adults, and on these grounds previous studies have confirmed the existence of "gut-muscle axis".

Although a gut-muscle axis might exist, the underlying pathogenesis driving frailty remains incompletely understood in particular the role of the gut microbiome driving the frailty phenotype. We hypothesise that frail older adults will have a lower IM diversity than the non-frail ones in the meta-analyses of previous studies. Furthermore, we expect to see differences in the relative abundance of bacteria.

2. Materials and methods

2.1 Protocol and registration

This systemic review was reported according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Guidelines (Moher et al., 2009). All details of the review protocol can be assessed on PROSPERO (CRD42021276733).

2.2 Search strategy and selection criteria

The syntax combined the synonyms for frailty (e.g. "sarcopenia" or "muscular atrophy" or "sarcopenic" or "physical frailty" or "barthel index" or "frailty" or "frailties" or "frailness" or "frailty syndrome") and gut microbiome (e.g. "gastrointestinal microbiome" or "gut microbiome" or "gastrointestinal microbiomes" or "gut microbiota" or "fecal microbiota composition" or "microbiota" or "gut bacteria"), and details of each search can be assessed on PROSPERO (CRD42021276733). The search was conducted on November 2021 on PubMed (MEDLINE), Embase, Cochrane and Web of Science. There was no restriction on the publication's date, and the terms were searched within all words in titles and abstracts.

Published articles were included in this meta-analysis if they met the following criteria: population older adults (\geq 60 years), both sexes, studies with comparison between two groups frail and non-frail (any validated scale); and the studies that measured diversity IM. The exclusion criteria encompassed non-original studies such as reviews, conference papers, letters and commentaries, non-human studies, non-English language, and studies that did not assess alpha diversity or relative abundance in frail and non-frail groups. Complementarily, papers that evaluated differences in the relative abundance in frail populations regarding the bacteria phyla, families, genera, and species were included in qualitative analysis.

2.4 Study selection

The selection of studies based on their abstracts was made on Rayyan (Ouzzani et al., 2016). The studies retrieved after this selection were scrutinized in a spreadsheet and clustered for each of the 3 main analysis. Two authors (HMA and JC) selected the studies independently, and the disagreements were solved with further discussion.

2.5 Data extraction

Data extraction was also done independently, and compared to avoid errors. The main IM diversity assessment used in the literature is alpha diversity, which is based on the total number of species, relative abundances of the species, or indices that combine these two dimensions (Lozupone and Knight, 2008). Thus, the two alpha indices, richness index and diversity index, and the relative abundance of bacteria phyla, families, genera, and species were extracted for analysis.

Mean, standard deviation (SD) and sample number (n) between group frail and non-frail for each outcome variable were collected for analysis. Median and interquartile

range (IQR) was replaced by mean and SD (SD=(IQR / 1.35), if SD was not provided in the original study (Hozo et al., 2005). The online software WebPlotDigitizer 4.5 was used to convert pixels to the specific units of measure and used when extraction of data from figures was needed.

Secondary data, such as age of participants, type of frailty criteria applied, frailty effect, method of IM diversity assessment, relative abundance in the frail and non-frail groups and significance level each study, were also collected for descriptive purposes and subgroup analysis.

2.6 Assessment of study quality

The quality of the studies was assessed for characterization purposes and was not an exclusion criterion. It was assessed by the Newcastle-Ottawa scale (NOS) (Wells et al., n.d.), which evaluates the selection of the study groups, the comparability of the groups, and the ascertainment of the exposure of interest for case-control and cohort studies, leading to a maximum of 9 points score.

2.7 Statistical analyses

The meta-analyses were performed using Comprehensive Meta-Analysis (CMA) software, version 3.3.070. We performed two meta-analyses for group comparisons, one for each selected outcome (richness index and diversity index). In these meta-analyses the effect size was calculated based on the standard mean difference (SMD) between frail and non-frail groups. When there was significant heterogeneity ($p \le 0.05$) we calculated the randomized effect, and when there was no significant heterogeneity (p > 0.05) we used fixed effects. Publication bias was analysed by the Egger test and a p-value of ≤ 0.05 was considered significant.

For species richness index and diversity index, we also ran subgroup analyses, applying the Q test to compare the SMD of studies assessing frailty by different criteria (the ones assessing by frailty criteria and the ones by Sarcopenia criteria), assessing IM diversity by different methods, and the SMD of studies assessing alpha diversity by different indexes.

3. Results

3.1 Study selection

The flowchart of the study selection is shown in **Figure 1**. One study comparing the IM between frailty and control was excluded due to the inclusion of some frail individuals in the control group (Ntemiri et al., 2017). Studies presenting regression coefficients for comparison between more than two groups (frail and non-frail) were excluded from meta-analysis (Ghosh et al., 2020; Jackson et al., 2016; Maffei et al., 2017), but maintained for the systematic review (Kang et al., 2021; Margiotta et al., 2021, 2020; Picca et al., 2020; Ponziani et al., 2021; Ticinesi et al., 2020, 2017; Zhang et al., 2020). Thus, 11 studies were included in the review and seven in the meta-analyses.

3.2 Characteristics of the studies

Table 1 shows the different methods of frailty assessment in the studies included, such as Fried's Frailty Phenotype score (Fried et al., 2001), Rockwood Clinical Frailty Scale (Rockwood et al., 2005), Rockwood Scale Modified (Joseph et al., 2016) and Groningen Frailty Indicator (GFI) (Steverink et al., 2001), and some sarcopeniaassessments including the operational definition elaborated in the SPRINTT project (Marzetti et al., 2018, 2015), Asian Working Group for Sarcopenia 2019 Consensus Report (Chen et al., 2020), European Consensus on Sarcopenia (Cruz-Jentoft et al., 2010; Janssen et al., 2004), Foundation for the National Institutes of Health (FNIH) Sarcopenia Project (Studenski et al., 2014), European Working Group on Sarcopenia in Older People 2 (Cruz-Jentoft et al., 2019), 34-item frailty index (FI34) (Kim et al., 2013), and Rockwood index (Singh et al., 2012). We meta-analysed 340 (161 frail and 179 nonfrail), above 60 years old (range 63-83), including 81.2% woman, 36.4% community dwellers, 45.4% older hospitalized/ nursing homes and 18.8% older adults suffering from chronic diseases, such as chronic kidney disease (CKD). Most of the studies (85.7%) only included participants that did not consume antibiotics in the past one month and belonged to the same demographic location (Italy). Six studies assessed richness index (Kang et al., 2021; Margiotta et al., 2020; Picca et al., 2020; Ponziani et al., 2021; Ticinesi et al., 2020, 2017), three assessed diversity index (Kang et al., 2021; Margiotta et al., 2020; Zhang et al., 2020), and ten assessed the relative abundance of bacteria species (Jackson et al., 2016; Kang et al., 2021; Maffei et al., 2017; Margiotta et al., 2021, 2020; Picca et al., 2020; Ponziani et al., 2021; Ticinesi et al., 2020; van Tongeren et al., 2005; Zhang et al., 2020).

3.3 Risk of bias

The majority of the studies included in the meta-analyses presented a moderate or high-quality score on NOS: four of them scored 6 (Margiotta et al., 2021, 2020; Ticinesi et al., 2017; Zhang et al., 2020) and the other four scored 7 (Kang et al., 2021; Picca et al., 2020; Ponziani et al., 2021; Ticinesi et al., 2020). Within the studies included in the qualitative analysis, two had a moderate quality score of 5 (Jackson et al., 2016; Maffei et al., 2017) and one scored 3 (van Tongeren et al., 2005) due to the lack of clarity on the selection of controlled participants. See details of the NOS assessment in **Supplementary tables 1 and 2**.

3.4 Evidence synthesis

The forest plots (**Figure 2**) showed no significant difference between frail and non-frail groups for any of the alpha diversity indexes: richness index (SMD=-0.147; 95% CI -0.394; 0.100, p=0.243) and diversity index (SMD=-0.033; 95% IC -0.315; 0.250; p=0.820). There was no significant heterogeneity (p >0.05) or inconsistency between studies, and the Egger tests suggested that there were no significant effects for publication bias in these meta-analyses (p >0.05). Complementarily, when the only study assessing kidney patients (Margiotta et al., 2020) was excluded, we confirmed the absence of significance between frail and non-frail groups for richness (SMD=-0.190, 95%CI=-0.467; 0.088, p=0.180) and diversity indexes (SMD=-0.291, 95%CI=-0.784; 0.202, p=0.248). Although, another study assessed cirrhotic patients was included for analysis.

We performed a subgroup analysis on the richness and diversity index, dividing the analysis between studies assessing frailty by conventional scales (i.e. Fried scale, Rockwood score, GFI, modified Rockwood score) and studies assessing frailty by sarcopenia evaluation (i.e. Asian Working Group for Sarcopenia 2019 Guidelines, European Consensus on Sarcopenia, Foundation for the National Institutes of Health sarcopenia project). There was also no difference in the subgroups of frailty assessment between frail and non-frail for the richness index (only frailty criteria SMD=-0.227; 95%IC=-0.578; 0.122; p=0.202; and by sarcopenia criteria SMD=-0.067; 95%IC=-0.416; 0.281; p=0.704, p difference [Q test] =0.525) and the diversity index (only frailty

evaluation SMD=0.017; 95%IC=-0.96; 0.331; p=0.913; and frailty by sarcopenia evaluation (SMD=-0.243; 95%IC=-0.887; 0.400; p=0.459, p difference [Q test] =0.476).

No difference between frail and non-frail was identified within the Chao 1 index (RMD=-17.385, 95%CI=-73.882; 39.113, p=0.546), the Index of species richness (RMD=-3.000, 95%CI=-25.049, 19.049, p=0.790), the Shannon index (RMD=0.075, 95%CI=-0.243; 0.394, p=0.643), the Simpson index (RMD=0.003, 95CI%=-0.009, 0.016; p=0.599), and Species diversity (RDM=-90.909, 95%CI=-331.259; 149.441, p=0.458).

The last subgroup analysis was the comparison between different IM composition assessments of the small ribosomal subunit (16S rRNA) gene, by regions V3-V4, only V3, shotgun metagenomic sequencing and the ones that did not inform the region. There was no difference in the effect of frailty on the diversity index (p=0.443) between the studies assessing 16S rRNA in the regions V3-V4 (SMD=-0.017, 95%CI=-0.433; 0.399, p=0.936) and the studies assessing 16S rRNA with no clear region defined (SMD=-0.358, 95%CI=-1.124; 0.408, p=0.360). There was also no difference in the effect of frailty on the richness index (p=0.391) among studies assessing 16S rRNA in V3 (SMD=-0.401, 95%CI=-0.858; 0.057, p=0.086), assessing 16S rRNA in V3-V4 (SMD=0.001, 95%CI=-0.347; 0.348, p=0.997) and study shotgun metagenomic sequencing (SMD=-0.146, 95%CI=-1.186; 0.902, p=0.790).

We presented in **Table 2** the p-value of the primary studies' comparisons between frail and non-frail, for the relative abundance of bacteria phyla, families, genera, or species. Frail group had a higher TM7 phylum; Barnesiellaceae, Bifidobacteriaceae, Mogibacteriaceae, *Micrococcaceae*, Coriobacteriaceae. Peptostreptococcaceae, Ruminococcaceae families: Actinomyces, Anaerotruncus, Bifidobacterium, Coprobacillus, Dialister, Dorea, Eggerthella, Erwinia, Eubacterium, Faecalibacterium, Lactobacillus, Megasphaera, Oscillospira, Rothia, Ruminococcus, Pyramidobacter Veillonella genera; and Eggerthella lenta, Eubacterium cylindroides, Eubacterium dolichun species than the non-frail group. The frail group had a lower relative abundance of Firmicutes and Verrucomicrobia phyla; Erysipelotrichaceae, Gemellaceae families; Acidaminococcus, Bacteroides, Prevotella, Fusicatenibacter, Gemella, Paraprevotella, Lachnoclostridium, Roseburia, Slackia, Sutterella genera; and Alistipes shahii, Faecalibacterium prausnitzii, Roseburia inulinivorans species than the non-frail group. The relative abundance of the Verrucomicrobiaceae, Veillonellaceae and Rikenellaceae

families, and the *Akkermansia genus* presented inconsistent results across the studies, being significantly higher in frail or in non-frail depending on the study that was analysed.

4. Discussion

On assessment of alpha diversity, no significant differences were observed between frail and non-frail groups. Diversity and richness index analyses were both consistent and homogeneous, suggesting that there was substantial evidence despite not many studies being included. Our biodiversity analyses were restricted to alpha diversity due to the absence of beta diversity in the studies included. Although there were no differences in IM alpha diversity between the frail and non-frail groups, it is important to highlight that alpha diversity indexes, such as the Chao 1 index (the most commonly used among the studies included), are based on the total number of species within a community. This means that they do not evaluate the dominance and equality of the microorganisms, and are unable to identify differences between species composition (Lozupone and Knight, 2008). With regards to beta diversity, the literature was also inconclusive as one study showed lower beta diversity for frail older adults compared to healthy older adults (Kang et al., 2021), whilst another study investigating IM beta diversity in hospitalized older adults found a higher beta diversity in frail older adults (Zhang et al., 2020). Although it is not expected that frail older adults have a higher beta diversity than non-frail older adults, this contradictory finding could be due to the hospitalization. This also could have been a source of theoretical heterogeneity in our alpha diversity meta-analysis, since the population of the studies included in our review consisted of 57.1% hospitalized older adults and 14.2 % CKD patients. In fact, changes in the composition of the IM of hospitalized patients have been associated with an altered dietary pattern, primarily driven by a low ingestion of dietary fibre, polypharmacy, and slowed transit time (Milani et al., 2016; Roager et al., 2016; Vaziri et al., 2013). Furthermore, studies that have compared alpha diversity in middle-aged communitydwelling adults have found lower diversity in frail adults as expected (Jackson et al., 2016; Maffei et al., 2017).

Among the relative abundances of different phylum between frail and non-frail older adults, *Firmicutes* and *Verrumicrobia* were lower in frail older adults and both phyla are dominant in the healthy human IM (Rinninella et al., 2019). Inversely, the relative abundance of the *TM7* phylum was higher in the frail group, and there is evidence of a correlation between this phylum and inflammatory mucosal diseases which encompasses

inflammatory bowel disease (Brinig et al., 2003; Kuehbacher et al., 2008), this bacterial is possibly involved in the inflammageing potential of the microbiota (Fransen et al., 2017; Jakobsson et al., 2015). In this way, these differences support a shift towards a state of dysbiosis in the IM of frail older adults.

With regards to the relative abundance of families between frail and non-frail, the Barnesiellaceae, Bifidobacteriaceae, *Coriobacteriaceae*, *Mogibacteriaceae*, Ruminococcaceae, and Veillonellaceae are families with higher relative abundance in frail older adults and other studies have shown an association between their abundance and frailty or diseases prevalence (Aho et al., 2019; Claesson et al., 2012; Haran et al., 2018; Li et al., 2021; Lin et al., 2018; Lourenço et al., 2018). The Mogibacteriaceae family is commonly associated with inflammation in other niches, such as the periodontal environment (Lourenço et al., 2018). An imbalance in the IM ecosystem of this family is evidently increased in the frail population, and it is positively associated with C-reactive protein levels, further strengthening the hypothesis of a link between microbiome changes, inflammageing and frailty (Margiotta et al., 2020). The Barnesiellaceae family has also been associated with increase systemic levels of pro-inflammatory cytokines, such as TNFa (Margiotta et al., 2021). The Bifidobacteriaceae family is composed of important probiotic bacteria that regulate intestinal and immune system functions, but since they were improved in the frail group we speculate this could be a potential compensatory mechanism to rebalance gut homeostasis (Wallen et al., 2020). Indeed, another study found higher relative abundance of the Bifidobacteriaceae family in patients with Parkinson's disease, who also have other markers of poor IM diversity (Shen et al., 2021).

With respect to the relative abundance of genera, *Actinomyces, Anaerotruncus, Coprobacillus, Dialister, Dorea, Eggerthella,* Eubacterium, *Rothia,* and *Veillonella* were higher in frail than non-frail older adults. Changes in the IM *Actinomyces* species are expected to influence various alimentary tract diseases and inflammation (Li et al., 2018). These diseases cause epithelial atrophy in the intestine, which can induce diminished mucosal resistance. *Anaerotruncus* and *Coprobacillus* have also been associated with inflammation and ageing in prior studies (Candela et al., 2014; Conley et al., 2016). Surprisingly, the *Bifidobacterium, Faecalibacterium, Lactobacillus, Ruminococcus,* and *Oscillospira* genera that are associated with a healthy gut and longevity (Biagi et al., 2016; Wang et al., 2015) were higher in frail older adults. In fact, previous studies have

shown that although Lactobacillus is considered probiotic due to its ability to regulate immune function, produce antioxidants (some species), adhere to the mucus layer in the gut to protect against the invasion of pathogens (Goldstein et al., 2015; Ljungh and Wadström, 2006), some species within this genus can be elevated under specific inflammatory conditions (Liu et al., 2013; Salminen et al., 2006).

An important finding of this review is that the increase in the relative abundance of the *Eggerthella* genus in frail older people was evidenced in four studies within this review (Jackson et al., 2016; Maffei et al., 2017; Margiotta et al., 2020; Picca et al., 2020). Bacteria from this genus use the amino acid threonine, the main component of intestinal mucin, deregulating intestinal epithelial junctions and increasing paracellular permeability to endotoxins (Rao, 2008). Additionally, *Eggerthella lenta*, a key species within the *Eggerthella* genus, was higher in frail older adults in one of the two studies that tested this species (Jackson et al., 2016; Margiotta et al., 2020). Although, *Eggerthella lenta* has been previously considered a commensal bacteria in the gut, it has been associated with gastrointestinal disease (Thota et al., 2011) and strongly correlated with inflammatory diseases (Zhang et al., 2015).

The relative abundance of the *Alistipes shahii, Roseburia inulinivorans*, and *Faecalibacterium prausnitzii* species was lower in frail than in non-frail. Those species are saccharolytic bacteria responsible for short-chain fatty acid (SCFA) generation (Parker et al., 2020). *Faecalibacterium prausnitzii* is considered a key butyrate producer (Sokol et al., 2008), and three studies showed significantly lower relative abundances with frailty. *Prevotella, Fusicatenibacter*, Lachnoclostridium, and *Roseburia* genera that are producers of SCFAs were also less abundant in frail older adults. SCFAs play an important role in IM health since butyrate is the main energy source for the colonic epithelial cells (Louis and Flint, 2017) and helps maintain tight junctions to regulate intestinal permeability (Peng et al., 2009), which in turn prevents endotoxin translocation and activation of inflammatory pathways (Vinolo et al., 2011), a potential anti-inflammageing effect. Furthermore, these fatty acids have an effect on muscle cells, improving mitochondrial activity, fatty acid oxidation (Vinolo et al., 2016; Saint-Georges-Chaumet and Edeas, 2016).

The relative abundance of the *Verrucomicrobiaceae and Veillonellaceae* families and the *Akkermansia* genus was higher in frail older adults in some studies but lower in frail older adults in other studies. Higher abundances of these families and genus have been associated with a healthy IM diversity, and thus we would expect to see lower abundances in frailty (Margiotta et al., 2021). It is possible that controversial increments in these potential commensal bacteria are an attempt of the IM to compensate intestinal dysfunction and dysbiosis in frail adults.

The findings of this study need to be interpreted with its limitations. Firstly, the small sample size of the studies and the specific ethnic origin (only from Italy, China, Netherlands, UK, USA) require for a cautious interpretation of results and impede generalization of findings older adults from other countries. Given the small number of studies, we could not test the influence of confounding factors such as diet, physical activity, co-morbid conditions, and medications. Polypharmacy and antibiotics, for example, are among the factors that are related to intestinal dysbiosis in older adults (Becattini et al., 2016; Ticinesi et al., 2017), and future studies should explore the interaction between drugs and IM diversity in frail older adults. Another limitation was the inclusion of studies assessing sarcopenia to represent frailty, since frailty is a comprehensive criterion. However, we compared the effect of frailty on IM diversity between these studies and no difference was found, suggesting the IM diversity is not affected by frailty when it is assessed by the validated frailty criteria or only by sarcopenia.

Also, despite the meta-analysis of diversity indexes had sample overlap caused by some studies that evaluated two types of indexes in the same population, we excluded this bias in the further subgroup analysis and confirmed the same results for each index without sample overlapping.

It is noteworthy that contradictory results of relative abundance for some bacteria species in different studies could have been influenced by different methods of IM composition assessments and more studies will be necessary to clarify this influence in future integrative analysis.

5. Conclusion

The relationship between microbiota and frailty is complex, and this has been further highlighted in our meta-analyses consistently found no difference in alpha diversity between frail and non-frail, there was some incipient evidence regarding the different relative abundance of bacteria between frail and non-frail older adults. Future studies need to consider the influence of many covariates, such as diet, location, physical activity level, multimorbidity, and polypharmacy, on the IM composition of frail individuals. The present results reinforce the need for further investigation on the role of specific bacteria and microbial metabolites and their influence on the physiopathology of frailty. Thus, the relationship between IM and frailty in older adults remains a very promising area of research and a target for closing the gap between lifespan and health span.

Declaration of Conflicts of Interest

None.

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Figure 1. Flowchart of the selection of the studies. Text boxes on the left describe the phase of the selection of the studies, while text boxes in the middle describe the studies retained in each phase, and the text boxes on the right describe the studies excluded.

 Table 1. Characteristics of the studies included.

First Author, Year	Participants	Age (Mean ± SD)	BMI (Mean ± SD)	Gende r	Country	N	Frailty criteria	Cut-off	Methods of IM assessment
Zhang, 2020	Hospitalized	81.63 (± 7.90)	F 21.07 (± 4.27) NF 23.83 (± 2.48)	Both	China	27	Modified Rockwood scale	F >0.25/ NF ≥ 0.25	16S rRNA sequencing without region information, for alpha diversity and relative abundance
Margiotta, 2020	CKD	F 81.8 (± 5.8) NF 79.03 (±6.6)	F 28.77 (± 5.4) NF 27.7 (± 3.4)	Both	Italy	64	Fried score	$F \ge 3 / NF < 3$	16S rRNA sequencing of V3-V4, for alpha diversity and relative abundance
Margiotta, 2021	CKD	S 83.1 (±5.7) NS 79.7 (±6.2)	S 25.5 (±2.6) NS) 29.3 (±4.8)	Both	Italy	63	EWGSOP2	S: HS <27 kg M and 16> kg W or 5XSST >15 seconds + ALM <20 kg M and <15 kg W or SMI <7.0 kg/m ² M and <5.5 kg/m ² + GS ≤ 0.8 m/s or SPPB ≤ 8 or TUG ≥ 20 s or 400WT non- completion or ≥ 6 min for completion	16S rRNA sequencing of V3-V4, for relative abundance
Picca, 2020	Community dwellers	FS 75.5 (±3.9) NFS 73.9 (± 3.2)	FS 32.14 5 (± 6.02) NFS 26.27 (± 2.55)	Both	Italy	35	SPRINTT project	FS: $3 \le \text{SPPB} \ge 9 + \text{ALM}$ (FNIH) + absence of mobility disability	16S rRNA sequencing of V3-V4, for alpha diversity and

									relative abundance
Ticinesi, 2020	Hospitalized	FS 77 (75.5–86)* NFS 71.5 (70–75)*	FS 24.3 (20.9– 26.7)* NFS 27.4 (24.5– 29.1)*	Both	Italy	17	SPPB + Muscle Mass (ECS)	FS: 3/12 and 9/12 SPPB + SMI ECS / NFS: 10/12 and 12/12 SPPB ou ECS	Shotgun metagenomic sequencing, for alpha diversity and relative abundance
Kang, 2021	Hospitalized	S 76.45 (± 8.58) NS 68.38 (± 5.79)	S 20.67 (± 3.27) NS 23.66 (± 2.49)	Both	China	71	AWGSG	S: HS <28 kg M and <18 kg W + 6MWT <1.0 m/s, SPPB \leq 9, or 5XSST \geq 12 seconds + SMI < 7.0 kg/m2 M or < 5.7 kg/m2 W	16S rRNA sequencing of V3-V4, for alpha diversity and relative abundance
Ponziani, 2021	Community- dwellers	S 75.5 (72- 77.25)* NS 72.5 (58.25- 75.25)*	S 29.99 (29- 31.79)* NS 26.2 (24.39- 28.68)*	Both	Italy	50	FNIH	ALMBMI < 0.789 M and < 0.512 W; or crude ALM < 19.75 kg M and < 15.02 kg W + HS < 26 kg M or < 16 kg W	16S rRNA sequencing of V3-V4, for alpha diversity and relative abundance
Van Tongeren, 2005	Nursing homes	86 (70 - 100)*	ND	Both	Netherland s	23	GFI	LF 1 – 4 / HF >5	Hybridization probes, for relative abundance
Ticinesi, 2017	Hospitalized	83.3 (± 7.5)	ND	Both	Italy	76	Rockwood scale	$F \le 7/NF \ge 4$	16S rRNA sequencing of V3, for alpha

diversity

Jackson, 2016	Community- dwellers	63 (42 -86) ND	Female	UK	728 Rockwood index	LF $\leq 0.25 / > 0.25$ MF $\leq 0.4 /$ HF > 0.4	16S rRNA sequencing of V4, for relative abundance
Maffei, 2017	Community- dwellers	63 (± 6)	Both	USA	85 FI34	LF 0-0.083/ MF 0.091-0.137/ HF 0.142-0.365	16S rRNA sequencing of V3-V4, for relative abundance

Legend: SD: ± standard; F: Frail; NF: Not frail; 16S rRNA: 16S Ribosomal Ribonucleic Acid; V3: region of the 16S rRNA gene; V3-V4: regions of the 16S rRNA gene; V4: regions of the 16S rRNA gene; CKD: Chronic Kidney Disease; S: Sarcopenic; NS: Not sarcopenic; EWGSOP2: European Working Group on Sarcopenia in Older People 2; HS: Handgrip strength; M: Men; W: Women; 5XSST: Five Times Sit to Stand Test; ALM: appendicular lean mass; SMI: Skeletal muscle index; GS: Gait speed; SPPB: Short-Physical Performance Battery; TUG: Timed Up and Go Test; WT: Walk test; FS: Frail and sarcopenic; NFS: Not frail and sarcopenic; +: associated with; FNIH: Foundation for the National Institutes of Health sarcopenia project; ECS: European Consensus on Sarcopenia; 6MWT: 6-minute walk test; AWGSG: Asian Working Group for Sarcopenia 2019 Guidelines; FNIH: Foundation for the National Institutes of Health; ALMBMI: appendicular lean mass to body mass index ratio; ND: No data; GFI: GFI; LF: Low frailty; HF: High frailty; UK: United Kingdom; MF: Middle frailty; USA: United States of America; FI₃₄: 34-item frailty index.



1.00

Frail

2.00



В										
Frist author, year	Subgroup	F	NF	SMD [LL;UL]	p-Value		Diversity inde	x SMD and 9	5% CI	
Margiotta, 2020	Simpson and Shannon	74	40	0.207 [-0.179; 0.592]	0.293			_+∎	-	
Zhang, 2020	Simpson and Shannon	30	24	-0.356 [-0.898; 0.185]	0.197			∎┼		
Kang, 2021	Species diversity	11	60	-0.243 [-0.887; 0.401]	0.459					
Summarized fixed effe	cts	115	124	-0.033 [-0.315; 0.250]	0.820		I	\bullet	•	
						-2.00	-1.00	0.00	1.00	2.00
Test for heterogeneity	: Q= 3.26; df= 2; p= 0.2; I ² =	38.7%;	Test fo	or overall effect z=-0.23 (p=0	0.82).		Non-frail		Frail	

Legend: F: frail; NF: non-frail; SMD: standard mean difference; LL: low limit; UL: upper limit; CI: confidence interval; ISR: Index of species richness. The text boxes below the forest plot tables detail the heterogeneity analyses and the test of overall hypothesis for each meta-analysis.

Phyla of bacteria	Frailty effect	p-value	Value F vs NF	Sample size F vs NF	Frailty criteria	Ref
Acidobacteria	F < NF	p > 0.05	0.2 vs 0.5 (M)	15 vs 12	Rockwood	Zhang, 2020
Actinobacteria	F < NF	p > 0.05	4.3 vs 4.4 (M)	15 vs 12	Rockwood	Zhang, 2020
Actinobacteria	F > NF	p > 0.05	0.6*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Actinobacteria	F < NF	p > 0.05	-0.6*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Bacteroidetes	F > NF	p > 0.05	22.8 vs 21.3 (M)	15 vs 12	Rockwood	Zhang, 2020
Bacteroidetes	F < NF	p > 0.05	-0.3*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Bacteroidetes	F < NF	p > 0.05	-0.1*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Chloroflexi	F < NF	p > 0.05	0.4 vs 0.7 (M)	15 vs 12	Rockwood	Zhang, 2020
Cyanobacteria	F > NF	p > 0.05	0.8*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Cyanobacteria	F > NF	p > 0.05	0.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Epsilonbacteraeota	F < NF	p > 0.05	0.4 vs 0.5 (M)	15 vs 12	Rockwood	Zhang, 2020
Euryarchaeota	F < NF	p > 0.05	-2.4*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Firmicutes	F > NF	p > 0.05	61 vs 60 (M)	15 vs 12	Rockwood	Zhang, 2020
Firmicutes	F > NF	p > 0.05	0.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Firmicutes	F < NF	p < 0.05	40.4 vs 54.4 (M)	11 vs 60	Sarcopenia	Kang, 2021
Firmicutes	F < NF	p > 0.05	-0.6*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Fusobacteria	F < NF	p > 0.05	0.6 vs 0.7 (M)	15 vs 12	Rockwood	Zhang, 2020
Patescibacteria	F > NF	p > 0.05	0.2 vs 0.03 (M)	15 vs 12	Rockwood	Zhang, 2020
Proteobacteria	F < NF	p > 0.05	9.5 vs 10.3 (M)	15 vs 12	Rockwood	Zhang, 2020
Proteobacteria	F > NF	p > 0.05	1.3*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Proteobacteria	F > NF	p > 0.05	1.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Synergistetes	F > NF	p > 0.05	4.3*(DAA)	18 vs 17	Sarcopenia	Picca, 2020

Table 2. Comparison between F and NF for the relative abundance of bacteria phyla, families, genera, and species.

TM7	F > NF	p > 0.05	0.8*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
TM7	F < NF	p > 0.05	-0.9*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
TM7	F > NF	p < 0.05	0.04 (FDR)	28 vs 30	FI34	Maffei, 2017
Verrucomicrobia	F < NF	p > 0.05	1.0 vs 1.1 (M)	15 vs 12	Rockwood	Zhang, 2020
Verrucomicrobia	F < NF	p > 0.05	-1.1*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Verrucomicrobia	F < NF	p < 0.05	-6.4*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Families of bacteria	Frailty effect	p-value	Value F vs NF	Sample size F vs NF	Frailty criteria	Ref
Acidaminobacteraceae	F < NF	p > 0.05	0 vs 0.0001	38 vs 26	Fried	Margiotta, 2020
Actinomycetaceae	F > NF	p > 0.05	0.1 vs 0.06	38 vs 26	Fried	Margiotta, 2020
Aerococcaceae	F > NF	p > 0.05	0.0017 vs 0.0015	38 vs 26	Fried	Margiotta, 2020
Alcaligenaceae	F < NF	p > 0.05	0.2 vs 0.3	38 vs 26	Fried	Margiotta, 2020
Alcaligenaceae	F < NF	p > 0.05	-0.04*(DDA)	18 vs 17	Sarcopenia	Picca, 2020
Anaeroplasmataceae	F < NF	p > 0.05	0.001 vs 0.16	38 vs 26	Fried	Margiotta, 2020
Aurantimonadaceae	F < NF	p > 0.05	0 vs 0.0009	38 vs 26	Fried	Margiotta, 2020
Bacteroidaceae	F < NF	p > 0.05	5.7 vs 6.1	38 vs 26	Fried	Margiotta, 2020
Bacteroidaceae	F < NF	p > 0.05	-0.17 *(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Bacteroidaceae	F > NF	p > 0.05	-0.4*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Barnesiellaceae	F < NF	p > 0.05	0.4 vs 0.5	38 vs 26	Fried	Margiotta, 2020
Barnesiellaceae	F > NF	p > 0.05	0.27 *(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Barnesiellaceae	F > NF	p < 0.05	2.5*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Bifidobacteriaceae	F > NF	p > 0.05	5.7 vs 3.6	38 vs 26	Fried	Margiotta, 2020
Bifidobacteriaceae	F > NF	p > 0.05	0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Bifidobacteriaceae	F > NF	p < 0.05	2.1*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Brevibacteriaceae	F < NF	p > 0.05	0.0003 vs 0.001	38 vs 26	Fried	Margiotta, 2020
Brucellaceae	F < NF	p > 0.05	0.0003 vs 0.0006	38 vs 26	Fried	Margiotta, 2020
Caldicoprobacteraceae	F < NF	p > 0.05	0 vs 0.0008	38 vs 26	Fried	Margiotta, 2020

Campylobacteraceae	F > NF	p > 0.05	0.003 vs 0.002	38 vs 26	Fried	Margiotta, 2020
Carnobacteriaceae	F < NF	p > 0.05	0.01 vs 0.02	38 vs 26	Fried	Margiotta, 2020
Carnobacteriaceae	F < NF	p > 0.05	-0.05	18 vs 17	Sarcopenia	Picca, 2020
Carnobacteriaceae	F < NF	p > 0.05	0.05*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Caulobacteraceae	F < NF	p > 0.05	0 vs 0.004	38 vs 26	Fried	Margiotta, 2020
Christensenellaceae	F < NF	p > 0.05	0.4 vs 0.9	38 vs 26	Fried	Margiotta, 2020
Christensenellaceae	F < NF	p > 0.05	-0.17 *(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Christensenellaceae	F < NF	p > 0.05	-1.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Clostridiaceae	F < NF	p > 0.05	3.8 vs 5.5	38 vs 26	Fried	Margiotta, 2020
Clostridiaceae	F > NF	p > 0.05	1.4 *(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Clostridiaceae	F < NF	p > 0.05	-0.05*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Comamonadaceae	F < NF	p > 0.05	0.0007 vs 0.01	38 vs 26	Fried	Margiotta, 2020
Coriobacteriaceae	F < NF	p > 0.05	-0.35	18 vs 17	Sarcopenia	Picca, 2020
Coriobacteriaceae	F < NF	p > 0.05	-0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Coriobacteriaceae	F > NF	p < 0.01	1.2 ± 0.3 vs 0.8 ± 0.1 (M)	38 vs 26	Fried	Margiotta, 2020
Coriobacteriaceae Corynebacteriaceae	F > NF $F > NF$	p < 0.01 p > 0.05	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005	38 vs 26 38 vs 26	Fried Fried	Margiotta, 2020 Margiotta, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae	F > NF F > NF F < NF	p < 0.01 p > 0.05 p > 0.05	1.2 ± 0.3 vs 0.8 ± 0.1 (M) 0.01 vs 0.005 0 vs 0.001	38 vs 26 38 vs 26 38 vs 26	Fried Fried Fried	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae	F > NF $F > NF$ $F < NF$ $F = NF$	p < 0.01 p > 0.05 p > 0.05 p > 0.05	1.2 ± 0.3 vs 0.8 ± 0.1 (M) 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011	38 vs 26 38 vs 26 38 vs 26 38 vs 26 38 vs 26	Fried Fried Fried Fried	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae	F > NF $F > NF$ $F < NF$ $F = NF$ $F > NF$	p < 0.01 p > 0.05 p > 0.05 p > 0.05 p > 0.05	1.2 ± 0.3 vs 0.8 ± 0.1 (M) 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA)	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17	Fried Fried Fried Fried Sarcopenia	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae	F > NF $F > NF$ $F < NF$ $F = NF$ $F > NF$ $F < NF$	p < 0.01 $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA) -1.1*(DAA)	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36	Fried Fried Fried Sarcopenia Sarcopenia	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae Dermabacteraceae	F > NF $F > NF$ $F < NF$ $F = NF$ $F > NF$ $F < NF$ $F < NF$	p < 0.01 p > 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA) -1.1*(DAA) 0.00004 vs 0.0001	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26	Fried Fried Fried Sarcopenia Sarcopenia Fried	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae Dermabacteraceae Desulfovibrionaceae	F > NF $F > NF$ $F < NF$ $F > NF$ $F > NF$ $F < NF$ $F < NF$ $F < NF$	p < 0.01 p > 0.05 p > 0.05	1.2 ± 0.3 vs 0.8 ± 0.1 (M) 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA) -1.1*(DAA) 0.00004 vs 0.0001 0.3 vs 0.4	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26	Fried Fried Fried Sarcopenia Sarcopenia Fried Fried	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae Dermabacteraceae Desulfovibrionaceae Desulfovibrionaceae	F > NF $F > NF$ $F < NF$ $F = NF$ $F < NF$	p < 0.01 p > 0.05 p > 0.05	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA) -1.1*(DAA) 0.00004 vs 0.0001 0.3 vs 0.4 1.8*(DAA)	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26 18 vs 17	Fried Fried Fried Sarcopenia Sarcopenia Fried Fried Sarcopenia	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Picca, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae Dermabacteraceae Desulfovibrionaceae Desulfovibrionaceae Desulfovibrionaceae	F > NF $F > NF$ $F < NF$ $F > NF$ $F < NF$ $F < NF$ $F < NF$ $F > NF$ $F > NF$ $F > NF$	p < 0.01 p > 0.05 p > 0.05	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA) -1.1*(DAA) 0.00004 vs 0.0001 0.3 vs 0.4 1.8*(DAA) 1.7*(DAA)	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26 18 vs 17 14 vs 36	Fried Fried Fried Sarcopenia Sarcopenia Fried Fried Sarcopenia Sarcopenia	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae Dermabacteraceae Desulfovibrionaceae Desulfovibrionaceae Desulfovibrionaceae Dethiosulfovibrionaceae	F > NF $F < NF$ $F = NF$ $F > NF$ $F < NF$ $F < NF$ $F < NF$ $F > NF$	p < 0.01 p > 0.05 p > 0.05	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA) -1.1*(DAA) 0.00004 vs 0.0001 0.3 vs 0.4 1.8*(DAA) 1.7*(DAA) 0.01 vs 0.002	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26	Fried Fried Fried Sarcopenia Sarcopenia Fried Fried Sarcopenia Sarcopenia Fried	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Picca, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae Dermabacteraceae Desulfovibrionaceae Desulfovibrionaceae Desulfovibrionaceae Dethiosulfovibrionaceae Dethiosulfovibrionaceae	F > NF $F > NF$ $F < NF$ $F > NF$ $F < NF$ $F < NF$ $F < NF$ $F > NF$	p < 0.01 p > 0.05 p > 0.05	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 0.24*(DAA) -1.1*(DAA) 0.00004 vs 0.0001 0.3 vs 0.4 1.8*(DAA) 1.7*(DAA) 0.01 vs 0.002 3.8*(DAA)	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17	Fried Fried Fried Sarcopenia Sarcopenia Fried Sarcopenia Sarcopenia Fried Sarcopenia Sarcopenia	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Picca, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Picca, 2020
Coriobacteriaceae Corynebacteriaceae Cytophagaceae Dehalobacteriaceae Dehalobacteriaceae Dehalobacteriaceae Dermabacteraceae Desulfovibrionaceae Desulfovibrionaceae Desulfovibrionaceae Dethiosulfovibrionaceae Elusimicrobiaceae	F > NF F > NF F < NF F = NF F > NF F < NF F < NF F > NF F	p < 0.01 p > 0.05 p > 0.05	$1.2 \pm 0.3 \text{ vs } 0.8 \pm 0.1 \text{ (M)}$ 0.01 vs 0.005 0 vs 0.001 0.010 vs 0.011 $0.24^*(\text{DAA})$ $-1.1^*(\text{DAA})$ 0.00004 vs 0.0001 0.3 vs 0.4 $1.8^*(\text{DAA})$ $1.7^*(\text{DAA})$ 0.01 vs 0.002 $3.8^*(\text{DAA})$ 0.002 vs 0	38 vs 26 38 vs 26 38 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17 38 vs 26	Fried Fried Fried Sarcopenia Sarcopenia Fried Sarcopenia Sarcopenia Fried Sarcopenia Fried Fried	Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Picca, 2020 Picca, 2020 Picca, 2020 Picca, 2020 Margiotta, 2020 Margiotta, 2020

Enterobacteriaceae	F > NF	p > 0.05	1.1*(DAA)
Enterobacteriaceae	F < NF	p > 0.05	5.3 vs 7.4
Enterobacteriaceae	F > NF	p > 0.05	1.3*(DAA)
Enterococcaceae	F < NF	p > 0.05	0.6 vs 0.7
Enterococcaceae	F > NF	p > 0.05	1.3*(DAA)
Enterococcaceae	F > NF	p > 0.05	1,9*(DAA)
Erysipelotrichaceae	F < NF	p > 0.05	3.8 vs 4.4
Erysipelotrichaceae	F < NF	p < 0.05	-2.4*(DAA)
Erysipelotrichaceae	F < NF	p > 0.05	-0.7*(DAA)
EtOH8	F < NF	p > 0.05	-0.07*(DAA)
Eubacteriaceae	F > NF	p > 0.05	0.02 vs 0.01
Flavobacteriaceae	F < NF	p > 0.05	0.00006 vs 0.0002
Fusobacteriaceae	F < NF	p > 0.05	0.01 vs 0.09
Gemellaceae	F < NF	p > 0.05	0.02 vs 0.03
Gemellaceae	F < NF	p < 0.05	0.042 (FDR)
Gemellaceae Geodermatophilaceae	F < NF $F < NF$	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001
Gemellaceae Geodermatophilaceae Gordoniaceae	F < NF F < NF F < NF	p < 0.05 p > 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae	F < NF $F < NF$ $F < NF$ $F < NF$	p < 0.05 p > 0.05 p > 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae	F < NF $F < NF$ $F < NF$ $F < NF$ $F > NF$	p < 0.05 $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae	F < NF $F < NF$ $F < NF$ $F > NF$ $F < NF$ $F < NF$	p < 0.05 $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$ $p > 0.05$	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae Lachnospiraceae	F < NF F < NF F < NF F > NF F < NF F > NF F > NF F > NF	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3 0.5*(DAA)
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lachnospiraceae	F < NF F < NF F < NF F > NF F < NF F > NF F	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3 0.5*(DAA) 2.9*(DAA)
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lachnospiraceae	F < NF F < NF F < NF F > NF	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3 0.5*(DAA) 2.9*(DAA) 2.3 vs 1.4
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lachospiraceae Lactobacillaceae	$F < NF \\ F < NF \\ F < NF \\ F > NF \\ F$	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3 0.5*(DAA) 2.9*(DAA) 2.3 vs 1.4 1.7*(DAA)
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lachospiraceae Lactobacillaceae Lactobacillaceae Lactobacillaceae	F < NF F < NF F < NF F > NF F	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3 0.5*(DAA) 2.9*(DAA) 2.3 vs 1.4 1.7*(DAA) 2.9*(DAA)
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lactobacillaceae Lactobacillaceae Lactobacillaceae Lactobacillaceae Lactobacillaceae	$F < NF \\ F < NF \\ F < NF \\ F > NF \\ F$	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3 0.5*(DAA) 2.9*(DAA) 2.3 vs 1.4 1.7*(DAA) 2.9*(DAA) 0.0006 vs 0.002
Gemellaceae Geodermatophilaceae Gordoniaceae Hyphomicrobiaceae Lachnospiraceae Lachnospiraceae Lachnospiraceae Lactobacillaceae Lactobacillaceae Lactobacillaceae Lactobacillaceae Lactobacillaceae Lactobacillaceae Lactobacillaceae	F < NF F < NF F < NF F > NF F	p < 0.05 p > 0.05	0.042 (FDR) 0 vs 0.0001 0 vs 0.0001 0.00006 vs 0.0002 22.8 vs 20.8 11.2 vs 11.3 0.5*(DAA) 2.9*(DAA) 2.3 vs 1.4 1.7*(DAA) 2.9*(DAA) 0.0006 vs 0.002 0.01 vs 0.02

18 vs 17	Sarcopenia	Picca, 2020
38 vs 26	Fried	Margiotta, 2020
14 vs 36	Sarcopenia	Ponziane, 2021
38 vs 26	Fried	Margiotta, 2020
18 vs 17	Sarcopenia	Picca, 2020
14 vs 36	Sarcopenia	Ponziane, 2021
38 vs 26	Fried	Margiotta, 2020
14 vs 36	Sarcopenia	Ponziane, 2021
18 vs 17	Sarcopenia	Picca, 2020
18 vs 17	Sarcopenia	Picca, 2020
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
18 vs 45	Sarcopenia	Margiotta, 2021
18 vs 45 38 vs 26	Sarcopenia Fried	Margiotta, 2021 Margiotta, 2020
18 vs 45 38 vs 26 38 vs 26	Sarcopenia Fried Fried	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020
18 vs 45 38 vs 26 38 vs 26 38 vs 26	Sarcopenia Fried Fried Fried	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
18 vs 45 38 vs 26	Sarcopenia Fried Fried Fried Fried	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
18 vs 45 38 vs 26 15 vs 12	Sarcopenia Fried Fried Fried Fried Rockwood	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Zhang, 2020
18 vs 45 38 vs 26 38 vs 26 38 vs 26 38 vs 26 15 vs 12 18 vs 17	Sarcopenia Fried Fried Fried Fried Rockwood Sarcopenia	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Zhang, 2020 Picca, 2020
18 vs 45 38 vs 26 38 vs 26 38 vs 26 38 vs 26 15 vs 12 18 vs 17 14 vs 36	Sarcopenia Fried Fried Fried Fried Rockwood Sarcopenia Sarcopenia	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Zhang, 2020 Picca, 2020 Ponziane, 2021
18 vs 45 38 vs 26 38 vs 26 38 vs 26 38 vs 26 15 vs 12 18 vs 17 14 vs 36 38 vs 26	Sarcopenia Fried Fried Fried Fried Rockwood Sarcopenia Sarcopenia Fried	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Zhang, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020
18 vs 45 38 vs 26 38 vs 26 38 vs 26 38 vs 26 15 vs 12 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17	Sarcopenia Fried Fried Fried Fried Rockwood Sarcopenia Sarcopenia Fried Sarcopenia	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Zhang, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Picca, 2020
18 vs 45 38 vs 26 38 vs 26 38 vs 26 38 vs 26 15 vs 12 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36	Sarcopenia Fried Fried Fried Fried Rockwood Sarcopenia Sarcopenia Fried Sarcopenia Sarcopenia	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Zhang, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Picca, 2020 Ponziane, 2021
18 vs 45 38 vs 26 38 vs 26 38 vs 26 38 vs 26 15 vs 12 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26	Sarcopenia Fried Fried Fried Fried Rockwood Sarcopenia Sarcopenia Fried Sarcopenia Fried Fried	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Picca, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020
18 vs 45 38 vs 26 38 vs 26 38 vs 26 38 vs 26 15 vs 12 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26	Sarcopenia Fried Fried Fried Fried Rockwood Sarcopenia Sarcopenia Fried Sarcopenia Fried Fried Fried	Margiotta, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Zhang, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020

Methanobacteriaceae	F > NF	p > 0.05	0.64*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Methanobacteriaceae	F < NF	p > 0.05	-1.8*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Methylobacteriaceae	F < NF	p > 0.05	0.0001 vs 0	38 vs 26	Fried	Margiotta, 2020
Microbacteriaceae	F < NF	p > 0.05	0.00007 vs 0.001	38 vs 26	Fried	Margiotta, 2020
Micrococcaceae	F < NF	p > 0.05	0.03 vs 0.06	38 vs 26	Fried	Margiotta, 2020
Micrococcaceae	F > NF	p < 0.05	FDR = 0.012	18 vs 45	Sarcopenia	Margiotta, 2021
Micrococcaceae	F < NF	p > 0.05	0.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Mogibacteriaceae	F > NF	p > 0.05	0.22 vs 0.19	38 vs 26	Fried	Margiotta, 2020
Mogibacteriaceae	F > NF	p < 0.05	0.2 ± 0.02 vs 0.02 ± 0.02 (M)	38 vs 26	Fried	Margiotta, 2020
Mogibacteriaceae	F < NF	p > 0.05	0.4*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Mogibacteriaceae	F < NF	p > 0.05	-0.5*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Moraxellaceae	F > NF	p > 0.05	0.0005 vs 0.0002	38 vs 26	Fried	Margiotta, 2020
Neisseriaceae	F > NF	p > 0.05	0.001 vs 0.0002	38 vs 26	Fried	Margiotta, 2020
Odoribacteraceae	F > NF	p > 0.05	0.4 vs 0.3	38 vs 26	Fried	Margiotta, 2020
Others	F < NF	p > 0.05	11 vs 11.2	38 vs 26	Fried	Margiotta, 2020
Oxalobacteraceae	F < NF	p > 0.05	0.004 vs 0.01	38 vs 26	Fried	Margiotta, 2020
Paraprevotellaceae	F > NF	p > 0.05	0.1 vs 0.06	38 vs 26	Fried	Margiotta, 2020
Paraprevotellaceae	F < NF	p > 0.05	-1.5*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Paraprevotellaceae	F < NF	p > 0.05	-1.9*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Pasteurellaceae	F < NF	p > 0.05	0.07 vs 0.3	38 vs 26	Fried	Margiotta, 2020
Pasteurellaceae	F > NF	p > 0.05	1.4*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Pasteurellaceae	F < NF	p > 0.05	-0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Peptococcaceae	F < NF	p > 0.05	0.03 vs 0.15	38 vs 26	Fried	Margiotta, 2020
Peptostreptococcaceae	F < NF	p > 0.05	0.9 vs 1.3	38 vs 26	Fried	Margiotta, 2020
Peptostreptococcaceae	F > NF	p > 0.05	1.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Peptostreptococcaceae	F > NF	p < 0.05	3*(DAA)	18 vs 17	Fried	Picca, 2020
Planococcaceae	F < NF	p > 0.05	0.01 vs 0.02	38 vs 26	Fried	Margiotta, 2020
Porphyromonadaceae	F > NF	p > 0.05	1 vs 0.8	38 vs 26	Fried	Margiotta, 2020

Porphyromonadaceae	F > NF	p > 0.05	0.17*(DAA)
Porphyromonadaceae	F < NF	p > 0.05	-0.5*(DAA)
Prevotellaceae	F < NF	p > 0.05	0.1 vs 0.2
Prevotellaceae	F < NF	p > 0.05	-1.6*(DAA)
Prevotellaceae	F < NF	p > 0.05	-1.4*(DAA)
Pseudomonadaceae	F < NF	p > 0.05	0.002 vs 0.03
Rhizobiaceae	F < NF	p > 0.05	0.0001 vs 0.01
Rhodobacteraceae	F > NF	p > 0.05	0.0001 vs 0
Rhodocyclaceae	F < NF	p > 0.05	0.00003 vs 0.0002
Rhodospirillaceae	F < NF	p > 0.05	0 vs 0.0002
Rikenellaceae	F > NF	p > 0.05	2.1 vs 1.4
Rikenellaceae	F > NF	p > 0.05	1.2*(DAA)
Rikenellaceae	F > NF	p < 0.05	2.2*(DAA)
Rikenellaceae	F < NF	p < 0.05	0.08 (FDR)
Ruminococcaceae	F < NF	p > 0.05	20 vs 20.4
		1	
Ruminococcaceae	F < NF	p > 0.05	-0.11*(DAA)
Ruminococcaceae Ruminococcaceae	F < NF F > NF	p > 0.05 p < 0.05	-0.11*(DAA) 1.2 *(DAA)
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae	F < NF $F > NF$ $F < NF$	p > 0.05 p < 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae	F < NF $F > NF$ $F < NF$ $F < NF$	p > 0.05 p < 0.05 p > 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae	F < NF $F > NF$ $F < NF$ $F < NF$ $F > NF$	p > 0.05 p < 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae Streptococcaceae	F < NF $F > NF$ $F < NF$ $F < NF$ $F > NF$ $F < NF$	p > 0.05 p < 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036 4.9 vs 5.7
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae Streptococcaceae Streptococcaceae	F < NF F > NF F < NF F < NF F < NF F < NF F < NF	p > 0.05 p < 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036 4.9 vs 5.7 -0.67*(DAA)
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae Streptococcaceae Streptococcaceae Streptococcaceae	F < NF F > NF F < NF F < NF F < NF F < NF F < NF F < NF	p > 0.05 p < 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036 4.9 vs 5.7 -0.67*(DAA) -0.2*(DAA)
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae	F < NF F > NF F < NF F > NF F < NF F < NF F < NF F < NF F < NF F < NF	p > 0.05 p < 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036 4.9 vs 5.7 -0.67*(DAA) -0.2*(DAA) 0 vs 0.0002
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptomycetaceae Succinivibrionaceae	F < NF F > NF F < NF	p > 0.05 p < 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036 4.9 vs 5.7 -0.67*(DAA) -0.2*(DAA) 0 vs 0.0002 0.0004 vs 0.01
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptomycetaceae Succinivibrionaceae Synergistaceae	F < NF F > NF F < NF	p > 0.05 p < 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036 4.9 vs 5.7 -0.67*(DAA) -0.2*(DAA) 0 vs 0.0002 0.0004 vs 0.01 0.036 vs 0.037
Ruminococcaceae Ruminococcaceae Sphingobacteriaceae Sphingomonadaceae Staphylococcaceae Streptococcaceae Streptococcaceae Streptococcaceae Streptomycetaceae Streptomycetaceae Succinivibrionaceae Synergistaceae Syntrophomonadaceae	F < NF F > NF F < NF	p > 0.05 p < 0.05 p > 0.05	-0.11*(DAA) 1.2 *(DAA) 0.00009 vs 0.0004 0.0002 vs 0.0009 0.038 vs 0.036 4.9 vs 5.7 -0.67*(DAA) -0.2*(DAA) 0 vs 0.0002 0.0004 vs 0.01 0.036 vs 0.037 0.00006 vs 0.0003

18 vs 17	Sarcopenia	Picca, 2020
14 vs 36	Sarcopenia	Ponziane, 2021
38 vs 26	Fried	Margiotta, 2020
18 vs 17	Sarcopenia	Picca, 2020
14 vs 36	Sarcopenia	Ponziane, 2021
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
38 vs 26	Fried	Margiotta, 2020
18 vs 17	Sarcopenia	Picca, 2020
14 vs 36	Sarcopenia	Ponziane, 2021
28 vs 30	FI34	Maffei, 2017
20 13 00		,
28 vs 26	Fried	Margiotta, 2020
38 vs 26 18 vs 17	Fried Sarcopenia	Margiotta, 2020 Picca, 2020
28 vs 26 38 vs 26 18 vs 17 14 vs 36	Fried Sarcopenia Sarcopenia	Margiotta, 2020 Picca, 2020 Ponziane, 2021
38 vs 26 18 vs 17 14 vs 36 38 vs 26	Fried Sarcopenia Sarcopenia Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020
38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26	Fried Sarcopenia Sarcopenia Fried Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020
28 vs 26 38 vs 26 18 vs 17 14 vs 36 38 vs 26	Fried Sarcopenia Sarcopenia Fried Fried Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
38 vs 26 18 vs 17 14 vs 36 38 vs 26	Fried Sarcopenia Sarcopenia Fried Fried Fried Fried Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 17	Fried Sarcopenia Sarcopenia Fried Fried Fried Fried Sarcopenia	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
38 vs 26 18 vs 17 14 vs 36 38 vs 26 18 vs 17 14 vs 36	Fried Sarcopenia Sarcopenia Fried Fried Fried Fried Sarcopenia Sarcopenia	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021
38 vs 26 18 vs 17 14 vs 36 38 vs 26	Fried Sarcopenia Sarcopenia Fried Fried Fried Fried Sarcopenia Sarcopenia Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020
38 vs 26 18 vs 17 14 vs 36 38 vs 26	Fried Sarcopenia Sarcopenia Fried Fried Fried Sarcopenia Sarcopenia Fried Fried Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020
38 vs 26 18 vs 17 14 vs 36 38 vs 26	Fried Sarcopenia Sarcopenia Fried Fried Fried Sarcopenia Sarcopenia Fried Fried Fried Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020
38 vs 26 18 vs 17 14 vs 36 38 vs 26 38 vs 26	Fried Sarcopenia Sarcopenia Fried Fried Fried Sarcopenia Sarcopenia Fried Fried Fried Fried Fried	Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Picca, 2020 Ponziane, 2021 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020 Margiotta, 2020

Tissierellaceae	F < NF	p > 0.05	0.046 vs 0.048	38 vs 26	Fried	Margiotta, 2020
Turicibacteraceae	F < NF	p > 0.05	0.1 vs 0.6	38 vs 26	Fried	Margiotta, 2020
Turicibacteraceae	F < NF	p > 0.05	-0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Veillonellaceae	F < NF	p > 0.05	1.3 vs 1.7	38 vs 26	Fried	Margiotta, 2020
Veillonellaceae	F > NF	p > 0.05	0.9*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Veillonellaceae	F > NF	p < 0.05	4.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Veillonellaceae	F < NF	p < 0.05	0.012 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Verrucomicrobiaceae	F > NF	p > 0.05	1.5 vs 0.8	38 vs 26	Fried	Margiotta, 2020
Verrucomicrobiaceae	F < NF	p > 0.05	-0.73*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Verrucomicrobiaceae	F < NF	p < 0.05	-3.7*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Verrucomicrobiaceae	F > NF	p < 0.05	0.012 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Vibrionaceae	F < NF	p > 0.05	0.002 vs 0.003	38 vs 26	Fried	Margiotta, 2020
Victivallaceae	F > NF	p > 0.05	0.01 vs 0.008	38 vs 26	Fried	Margiotta, 2020
Weeksellaceae	F < NF	p > 0.05	0.00009 vs 0.0002	38 vs 26	Fried	Margiotta, 2020
Xanthobacteraceae	F < NF	p > 0.05	0.00008 vs 0.0002	38 vs 26	Fried	Margiotta, 2020
Xanthomonadaceae	F < NF	p > 0.05	0.0001 vs 0.002	38 vs 26	Fried	Margiotta, 2020
Genera of bacteria	Frailty effect	p-value	Value F vs NF	Sample size F vs NF	Frailty criteria	Ref
Acidaminococcus	F < NF	p < 0.05	< 0.01 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Actinomyces	F > NF	p < 0.05	0.1 ± 0.01 vs 0.05 ± 0.01 (M)	38 vs 26	Fried	Margiotta, 2020
Adlercreutzia	F > NF	p > 0.05	0.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Adlercreutzia	F < NF	p > 0.05	-0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Akkermansia	F < NF	p > 0.05	-1.3*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Akkermansia	F < NF	p < 0.05	-4.4*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Akkermansia	F > NF	p < 0.05	0.008 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Alistipes	F > NF	p > 0.05	5.3 vs 5.0 (M)	15 vs 12	Rockwood	Zhang, 2020
Alistings	F > NF	n > 0.05	3.2 vs 0.8 (M)	11 vs 60	Sarconenia	Kang 2021

Anaerostipes	F < NF	p > 0.05	-0.04*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Anaerostipes	F < NF	p > 0.05	-1.1*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Anaerotruncus	F > NF	p < 0.01	0.04 ± 0.01 vs 0.01 ± 0.009 (M)	38 vs 26	Fried	Margiotta, 2020
Anaerotruncus	F < NF	p > 0.05	-1.0*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Atopobium	F > NF	p > 0.05	10.6 vs 3.4 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Atopobium	F > NF	p > 0.05	1.3*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Atopobium	F > NF	p > 0.05	0.9*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Bacteroides	F < NF	p > 0.05	35.9 vs 36.3 (M)	15 vs 12	Rockwood	Zhang, 2020
Bacteroides	F > NF	p > 0.05	38.9 vs 34.7 (M)	11 vs 60	Sarcopenia	Kang, 2021
Bacteroides	F < NF	p > 0.05	-0.5*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Bacteroides	F > NF	p > 0.05	0.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Bacteroides/Prevotella	F < NF	p < 0.05	9.4 vs 24.2 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Bifidobacterium	F > NF	p > 0.05	1.3 vs 0.5 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Bifidobacterium	F < NF	p > 0.05	4.6 vs 5.4 (M)	15 vs 12	Rockwood	Zhang, 2020
Bifidobacterium	F > NF	p > 0.05	7.6 vs 4.1 (M)	11 vs 60	Sarcopenia	Kang, 2021
Bifidobacterium	F > NF	p < 0.05	1.7*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Bifidobacterium	F > NF	p > 0.05	0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Bilophila	F > NF	p > 0.05	0.7*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Bilophila	F > NF	p > 0.05	2.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Blautia	F > NF	p > 0.05	7.9 vs 7.7 (M)	15 vs 12	Rockwood	Zhang, 2020
Blautia	F < NF	p > 0.05	3.0 vs 6.2 (M)	11 vs 60	Sarcopenia	Kang, 2021
Blautia	F < NF	p > 0.05	-0.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Blautia	F < NF	p > 0.05	-1.0*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Catenibacterium	F < NF	p > 0.05	-4.1*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Christensenella	F < NF	p > 0.05	-0.4*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Christensenella	F < NF	p > 0.05	-0.4*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Christensenellaceae_r-7_	F > NF	NR	1.7 vs 1.1 (M)	11 vs 60	Sarcopenia	Kang, 2021
Clostridium	F > NF	p > 0.05	0.2 vs 0.02 (MD)	10 vs 13	GFI	Van Tongeren, 2005

Collinsella	F > NF	p > 0.05	0.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Collinsella	F > NF	p > 0.05	0.1*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Coprobacillus	F > NF	p < 0.05	0.3 ± 0.1 vs 0.06 ± 0.01	38 vs 26	Fried	Margiotta, 2020
Coprobacillus	F > NF	p < 0.05	0.01 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Coprobacillus	F > NF	p < 0.05	0.08 (FDR)	28 vs 30	FI34	Maffei, 2017
Coprococcus	F > NF	p > 0.05	0.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Coprococcus	F < NF	p > 0.05	-0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Dehalobacterium	F > NF	p > 0.05	3.98E-05*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Dehalobacterium	F < NF	p > 0.05	-1.6*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Dialister	F > NF	p < 0.05	3,5*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Dialister	F > NF	p < 0.05	0.08 (FDR)	28 vs 30	FI34	Maffei, 2017
Dialister	F > NF	p > 0.05	2.8*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Dorea	F > NF	p < 0.05	8.9 ± 0.2 vs 8.1 ± 0.2 (M)	38 vs 26	Fried	Margiotta, 2020
Dorea	F < NF	p > 0.05	-0.7*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Dorea	F > NF	p > 0.05	0.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Eggerthella	F > NF	p < 0.01	0.2 ± 0.04 vs 0.08 ± 0.01 (M)	38 vs 26	Fried	Margiotta, 2020
Eggerthella	F > NF	p < 0.05	2.0*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Eggerthella	F > NF	p < 0.05	< 0.01 (FDR)	ND	Rockwood	Jackson, 2016
Eggerthella	F > NF	p < 0.05	<0.05 (FDR)	ND	FI34	Maffei, 2017
Eggerthella	F < NF	p > 0.05	-0.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Enterococcus	F > NF	p > 0.05	1.3*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Enterococcus	F > NF	p > 0.05	1.9*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Erwinia	F > NF	p < 0.01	0.06 ± 0.02 vs 0.006 ± 0.001	38 vs 26	Fried	Margiotta, 2020
Escherichia -Shigella	F > NF	p > 0.05	7.4 vs 3.1 (M)	11 vs 60	Sarcopenia	Kang, 2021
Eubacterium	F > NF	p < 0.05	-3,2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Eubacterium	F > NF	p > 0.05	-2.7*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Eubacterium	F > NF	p > 0.05	0.6 (FDR)	ND	Rockwood	Jackson, 2016
Faecalibacterium	F < NF	p > 0.05	7.3 vs 8.5 (M)	15 vs 12	Rockwood	Zhang, 2020

Faecalibacterium	F < NF	p > 0.05	5.0 vs 10.4 (M)	11 vs 60	Sarcopenia	Kang, 2021
Faecalibacterium	F < NF	p > 0.05	-0.9*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Faecalibacterium	F > NF	p > 0.05	1.8*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Faecalibacterium	F > NF	p < 0.05	< 0.01 (FDR)	ND	Rockwood	Jackson, 2016
Fusicatenibacter	F < NF	p < 0.05	0.5 vs 3.1 (M)	11 vs 60	Sarcopenia	Kang, 2021
Gemella	F < NF	p < 0.05	0.03 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Granulicatella	F > NF	p > 0.05	0.1*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Granulicatella	F < NF	p > 0.05	-0.8*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Haemophilus	F > NF	p > 0.05	1.4*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Haemophilus	F > NF	p > 0.05	0.4*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Klebsiella	F < NF	p > 0.05	-0.9*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Lachnobacterium	F < NF	p > 0.05	-1.3*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Lachnobacterium	F < NF	p > 0.05	-2.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Lachnoclostridium	F > NF	p > 0.05	8.2 vs 7.0 (M)	15 vs 12	Rockwood	Zhang, 2020
Lachnoclostridium	F < NF	p < 0.05	1.2 vs 2.2 (M)	11 vs 60	Sarcopenia	Kang, 2021
Lachnospira	F > NF	p > 0.05	0.2 vs 0.01 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Lachnospira	F < NF	p > 0.05	0.6 vs 2.7 (M)	11 vs 60	Sarcopenia	Kang, 2021
Lachnospira	F < NF	p > 0.05	-0.7*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Lachnospira	F < NF	p > 0.05	-0.6*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Lactobacillus	F > NF	p < 0.05	2.3 ± 0.6 vs 1.3 ± 0.5 (M)	38 vs 26	Fried	Margiotta, 2020
Lactobacillus	F > NF	p < 0.05	4.4 vs 0.7 (M)	11 vs 60	Sarcopenia	Kang, 2021
Lactobacillus	F > NF	p > 0.05	2.6*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Lactobacillus	F > NF	p < 0.05	4.7*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Lactobacillus/Enterococcus	F > NF	p < 0.01	0.3 vs 0.04 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Megasphaera	F > NF	p < 0.05	< 0.01 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Megamonas	F < NF	p > 0.05	1.6 vs 2.9 (M)	11 vs 60	Sarcopenia	Kang, 2021
Methanobrevibacter	F > NF	p > 0.05	1.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Methanobrevibacter	F < NF	p > 0.05	-1.8*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021

Oscillospira	F > NF	p < 0.05	$0.9 \pm 0.06 \text{ vs} \ 0.8 \pm 0.08 \text{ (M)}$	38 vs 26	Fried	Margiotta, 2020
Oscillospira	F > NF	p > 0.05	0.5*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Oscillospira	F > NF	p > 0.05	0.6*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Other	F > NF	p < 0.05	3.1*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Parabacteroides	F > NF	p > 0.05	6.3 vs 5.8 (M)	15 vs 12	Rockwood	Zhang, 2020
Parabacteroides	F > NF	p > 0.05	6.5 vs 2.4 (M)	11 vs 60	Sarcopenia	Kang, 2021
Parabacteroides	F < NF	p > 0.05	-0.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Parabacteroides	F < NF	p > 0.05	-0.02*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Paraprevotella	F < NF	p > 0.05	-1.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Paraprevotella	F < NF	p < 0.05	0.02 (FDR)	28 vs 30	FI34	Maffei, 2017
Paraprevotella	F < NF	p > 0.05	-0.7*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Phascolarctobacterium	F < NF	p > 0.05	0.01 vs 0.04 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Phascolarctobacterium	F < NF	p > 0.05	1.1 vs 1.3 (M)	11 vs 60	Sarcopenia	Kang, 2021
Phascolarctobacterium	F > NF	p > 0.05	0.9*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Phascolarctobacterium	F > NF	p > 0.05	3.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Prevotella	F < NF	p > 0.05	-1.6*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Prevotella	F < NF	p > 0.05	-1.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Prevotella_9	F < NF	p > 0.05	8.3 vs 10.1 (M)	11 vs 60	Sarcopenia	Kang, 2021
Pyramidobacter	F > NF	p < 0.05	4,5*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Roseburia	F < NF	p > 0.05	5.7 vs 7.0 (M)	15 vs 12	Rockwood	Zhang, 2020
Roseburia	F < NF	p < 0.05	0.8 vs 3.6 (M)	11 vs 60	Sarcopenia	Kang, 2021
Roseburia	F < NF	p > 0.05	-0.02*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Roseburia	F < NF	p > 0.05	-0.08*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Rothia	F < NF	p > 0.05	-0.7*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Rothia	F > NF	p < 0.05	< 0.01 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Ruminococcaceae_UCG-002	F > NF	p > 0.05	2.4 vs 0.8 (M)	11 vs 60	Sarcopenia	Kang, 2021
Ruminococcus	F > NF	p < 0.05	< 0.05 (FDR)	ND	FI34	Maffei, 2017
Ruminococcus	F < NF	p > 0.05	15.2 vs 23.8 (MD)	10 vs 13	GFI	Van Tongeren, 2005

Ruminococcus	F > NF	p > 0.05	1.8 vs 1.3 (M)	11 vs 60	Sarcopenia	Kang, 2021
Ruminococcus	F > NF	p > 0.05	0.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
[Ruminococcus]	F > NF	p > 0.05	0.6*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Ruminococcus¬	F > NF	p > 0.05	1.2*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Ruminococcus°	F > NF	p > 0.05	0.5*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Slackia	F < NF	p < 0.05	-7.2*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Slackia	F < NF	p < 0.05	-9.1*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Streptococcus	F > NF	p > 0.05	1.1 vs 0.8 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Streptococcus	F > NF	p > 0.05	7.5 vs 5.8 (M)	15 vs 12	Rockwood	Zhang, 2020
Streptococcus	F < NF	p > 0.05	-0.5*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Streptococcus	F < NF	p > 0.05	-0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Subdoligranulum	F <nf< td=""><td>p > 0.05</td><td>$0.17 \pm 0.3 \text{ vs} \ 0.21 \pm 0.2 \text{ (M)}$</td><td>5 vs 12</td><td>Sarcopenia</td><td>Ticinesi, 2020</td></nf<>	p > 0.05	$0.17 \pm 0.3 \text{ vs} \ 0.21 \pm 0.2 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Subdoligranulum	F < NF	p > 0.05	2.0 vs 2.6 (M)	11 vs 60	Sarcopenia	Kang, 2021
Sutterella	F < NF	p > 0.05	-0.4*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Sutterella	F < NF	p < 0.05	0.08 (FDR)	28 vs 30	FI34	Maffei, 2017
Turicibacter	F < NF	p > 0.05	-0.3*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Veillonella	F = NF	p > 0.05	0.01 vs 0.01 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Veillonella	F > NF	p > 0.05	2.4*(DAA)	18 vs 17	Sarcopenia	Picca, 2020
Veillonella	F > NF	p > 0.05	3.04*(DAA)	14 vs 36	Sarcopenia	Ponziane, 2021
Veillonella	F > NF	p < 0.05	< 0.01 (FDR)	18 vs 45	Sarcopenia	Margiotta, 2021
Species of bacteria	Frailty effect	p-value	Value F vs NF	Sample size F vs NF	Frailty criteria	Ref
Akkermansia muciniphila	F = NF	p > 0.05	$0 \pm 6.4 \text{ vs } 0 \pm 0.07 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Alistipes shahii	F < NF	p < 0.05	0 ± 0.15 vs 0.9 ± 1.1 (M)	5 vs 12	Sarcopenia	Ticinesi, 2020
Alistipes onderdonkii	F < NF	p > 0.05	$0.3 \pm 9.1 \text{ vs } 0.6 \pm 0.9 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Bacteroides caccae	F < NF	p > 0.05	$0.4 \pm 4.0 \text{ vs } 1.0 \pm 1.7 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Bacteroides dorei	F < NF	p > 0.05	$0.2 \pm 0.5 \text{ vs} \ 0.5 \pm 1.3 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020

Bacteroides fragilis	F > NF	p > 0.05	$0.4 \pm 8.4 \text{ vs} \ 0.3 \pm 1.1 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Bacteroides uniformis	F > NF	p > 0.05	13.0 ± 10.2 vs 6.3 ± 9.3 (M)	5 vs 12	Sarcopenia	Ticinesi, 2020
Bacteroides vulgatus	F < NF	p > 0.05	$1.7 \pm 1.6 \text{ vs } 3.8 \pm 5.7 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Barnesiella intestinihominis	F < NF	p > 0.05	$0.1 \pm 2.7 \text{ vs } 2.4 \pm 2.0 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Bifidobacterium longum	F > NF	p > 0.05	$0.4 \pm 0.6 \text{ vs} \ 0 \pm 0.3 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Eggerthella lenta	F > NF	p > 0.05	$5.1 \pm 0.3 \text{ vs} 4.0 \pm 0.3 \text{ (M)}$	38 vs 26	Fried	Margiotta, 2020
Eggerthella lenta	F > NF	p < 0.05	< 0.01 (FDR)	ND	Rockwood	Jackson, 2016
E. faecium/E. faecalis	F > NF	p > 0.05	0.01 vs 0.02 (MD)	10 vs 13	GFI	Van Tongeren, 2005
E. rectale/C. coccoides	F < NF	p > 0.05	13.2 vs 19.7 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Escherichia coli	F > NF	p > 0.05	$0.3 \pm 2.8 \text{ vs} \ 0 \pm 0.2 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Eubacterium cylindroides	F > NF	p < 0.05	6.0 ± 0.3 vs 5.0 ± 0.2 (M)	38 vs 26	Fried	Margiotta, 2020
Eubacterium cylindroides	F > NF	p > 0.05	1.4 vs 0.9 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Eubacterium dolichun	F > NF	p < 0.05	6.5 ± 0.3 vs 5.0 ± 0.4 (M)	38 vs 26	Fried	Margiotta, 2020
Eubacterium dolichun	F > NF	p < 0.05	< 0.01 (FDR)	ND	Rockwood	Jackson, 2016
Eubacterium hallii	F = NF	p > 0.05	0.1 vs 0.1 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Faecalibacterium prausnitzi	F < NF	p < 0.05	0.2 ± 2.8 vs 5.6 ± 6.0 (M)	5 vs 12	Sarcopenia	Ticinesi, 2020
Faecalibacterium prausnitzi	F < NF	p < 0.05	0.7 vs 3.1 (MD)	10 vs 13	GFI	Van Tongeren, 2005
Faecalibacterium prausnitzi	F < NF	p < 0.05	< 0.01 (FDR)	ND	Rockwood	Jackson, 2016
Flavonifractor plautii	F > NF	p > 0.05	$0.9 \pm 0.6 \text{ vs} \ 0.5 \pm 0.5 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Parabacteroides distasonis	F > NF	p > 0.05	$2.9 \pm 12.4 \text{ vs } 1.0 \pm 2.4 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Parabacteroides merdae	F > NF	p > 0.05	1.2 ± 2.2 vs 1.1 ± 1.5 (M)	5 vs 12	Sarcopenia	Ticinesi, 2020
Roseburia intestinalis	F < NF	p > 0.05	$0.2 \pm 0.3 \text{ vs} \ 0.3 \pm 1.2 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Roseburia inulinivorans	F < NF	p < 0.05	0.0 ± 0.0 vs 0.3 ± 0.6 (M)	5 vs 12	Sarcopenia	Ticinesi, 2020
Ruminococcus bromii	F > NF	p > 0.05	$0.9 \pm 1.1 \text{ vs} \ 0.3 \pm 1.0 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Ruminococcus gnavus	F > NF	p > 0.05	$0.3 \pm 2.4 \text{ vs} \ 0.1 \pm 0.2 \text{ (M)}$	5 vs 12	Sarcopenia	Ticinesi, 2020
Ruminococcus torques	F > NF	p > 0.05	$7.4 \pm 0.3 \text{ vs} \ 7.3 \pm 0.2 \text{ (M)}$	38 vs 26	Fried	Margiotta, 2020

Legend: *: Log2FC; DDA: Differential Abundance Analysis; M: Mean; ±: Standard Deviation; MD: Median; []: Non cited in study; ¬: Firmicutes, Clostridia, Clostridiales, Lachnospiraceae, Ruminococcus; °: Firmicutes, Clostridia, Ruminococcaceae, Ruminococcus; GFI: Groningen

Frailty Indicator; E. faecium: Enterococcus Faecium; E. faecalis: Enterococcus faecalis; E. rectale: Eubacterium Rectale; C. coccoides: Clostridium Coccoides. Bold lines have a level of significance p < 0.05 or p < 0.01.

First author, year	Selection				Comparability	Comparability Exposure				
	1	<mark>2</mark>	<mark>3</mark>	<mark>4</mark>	<mark>5</mark>	<mark>6</mark>	7	8		
Zhang, 2020	*	-	-	*	<mark>* *</mark>	*	*	-	<mark>6</mark>	
Margiotta, 2020	*	-	-	*	<mark>* *</mark>	*	*	-	<mark>6</mark>	
Margiotta, 2021	*	-	-	*	<mark>* *</mark>	*	*	-	<mark>6</mark>	
Picca, 2020	*	-	*	*	<mark>* *</mark>	*	*	-	<mark>7</mark>	
Ticinesi, 2020	*	-	*	*	<mark>**</mark>	*	*	-	<mark>7</mark>	
Kang, 2021	*	-	*	<mark>*</mark>	<mark>* *</mark>	*	*	-	<mark>7</mark>	
Ponziani, 2021	*	-	*	*	<mark>* *</mark>	*	*	-	<mark>7</mark>	
Van Tongeren, 2005	*	-	-	-	- T	*	*	-	<mark>3</mark>	
Ticinesi, 2017	*	-	_	*	<mark>* *</mark>	*	*	_	<mark>6</mark>	

Supplementary table 1. Newcastle-Ottawa assessment scale case control studies.

Legend: *: one point attributed in the question; **: two points attributed in the question; -: none point attributed in the question; 1: Is the case definition adequate?; 2: Representativeness of the cases; 3: Selection of Controls; 4: Definition of Controls; 5: Comparability of cases and controls on the basis of the design or analysis; 6: Ascertainment of exposure; 7: Same method of ascertainment for cases and controls; 8: Non-Response rate.

First author, year	Selection	<mark>n</mark>			Comparability	Exposure	<mark>Total</mark>		
	1	<mark>2</mark>	<mark>3</mark>	<mark>4</mark>	<mark>5</mark>	<mark>6</mark>	7	<mark>8</mark>	
Jackson, 2016	<mark>*</mark>	*	*	-	*	*	-	-	<mark>5</mark>
Maffei, 2017	*	*	*	-	*	*	-	-	<mark>5</mark>

Supplementary table 2. Newcastle-Ottawa Scale assessment cohort studies scale studies.

Legend: *: one point attributed in the question; **: two points attributed in the question; -: none point attributed in the question; 1: Representativeness of the exposed cohort; 2: Selection of the non-exposed cohort; 3: Ascertainment of exposure; 4: Demonstration that outcome of interest was not present at start of study; 5: Comparability of cohorts on the basis of the design or analysis; 6: Assessment of outcome; 7: Was follow-up long enough for outcomes to occur; 8: Adequacy of follow up of cohorts.