Table 4 Logistic regression analysis of frail condition among Japanese, Brazilian Japanese descendants and Brazilian participants using Kihon Checklist scores as dependent variables and nationality as covariate – adjusted by age

	Frailty % (*n*)	*P*	OR (95% CI)	*P*
Total KCL score (cut-off >6 points)		<0.001		
Japanese (reference for OR)	16.7 (14)		1	
Brazilian Japanese descendants	10.9 (6)		0.65 (0.23–1.84)	0.417
Brazilian	45.8 (33)		5.97 (2.69–13.3)	<0.001
IADL domain (cut-off >2 points)		0.194		
Japanese (reference for OR)	1.2 (1)		1	
Brazilian Japanese descendants	0		–	–
Brazilian	4.2 (3)		5.15 (0.51–52.2)	0.165
Physical strength domain		0.242		
Japanese (reference for OR)	21.4 (18)		1	
Brazilian Japanese descendants	10.9 (6)		0.44 (0.16–1.22)	0.114
Brazilian	20.8 (15)		0.95 (0.42–2.13)	0.892
Nutrition domain (cut-off BMI<20.5)		0.090		
Japanese (reference for OR)	6 (5)		1	
Brazilian Japanese descendants	1.9 (1)		0.22 (0.018–2.57)	0.226
Brazilian	0		–	
Eating domain		<0.001		
Japanese (reference for OR)	19 (16)		1	
Brazilian Japanese descendants	9.1 (5)		0.45 (0.15–1.33)	0.148
Brazilian	37.5 (27)		3.18 (1.47–6.85)	0.003
Socialization Domain (cut-off >1 point)		<0.001		
Japanese (reference for OR)	8.3 (7)		1	
Brazilian Japanese descendants	18.2 (10)		2.70 (0.95–7.73)	0.063
Brazilian	37.5 (27)		9.15 (3.53–23.7)	<0.001
Memory domain		<0.001		
Japanese (reference for OR)	29.8 (25)		1	
Brazilian Japanese descendants	38.2 (21)		1.49 (0.72–3.08)	0.279
Brazilian	61.1 (44)		3.87 (1.93–7.75)	<0.001
Mood domain		<0.001		
Japanese (reference for OR)	10.7 (9)			
Brazilian Japanese descendants	9.1 (5)		0.89 (0.28–2.83)	0.844
Brazilian	38.9 (28)		6.63 (2.74–16.0)	<0.001

Values represent percentage (*n*) and OR (95% CI); $n = 211$. BMI, body mass index; IADL, instrumental activities of daily living; KCL, Kihon Checklist.

differences regarding IADL performance, and physical and nutritional conditions among the groups (Table 4).

The results of the logistic regression confirmed that older Brazilian women were more inclined to be frail than Japanese women. The Brazilian participants were fivefold more likely to be frail (OR 5.97, 95% CI 2.69–13.3, $P < 0.001$), threefold more likely to have oral dysfunction (OR 3.18, 95% CI 1.47–6.85, $P = 0.003$), ninefold more likely to have seclusion (OR 9.15, 95% CI 3.53–23.7, $P < 0.001$), threefold more likely to have cognitive impairment (OR 3.87, 95% CI 1.93–7.75, $P < 0.001$) and sixfold more likely to have depression (OR 6.63, 95% CI 2.74–16.0, $P < 0.001$) than the older Japanese women. However, no difference was found between the Japanese and Brazilian Japanese descendants. No difference was found in terms of IADL, physical or nutritional domains among the groups (Table 4).

Discussion

In the present study, we observed a higher prevalence of frail participants in the Brazilian group ($P < 0.001$); and that older Brazilian women were more inclined to be frail than Japanese women (OR 5.97, 95% CI 2.87–13.3, $P < 0.001$). To the best of our knowledge, the present study is the first that compares frailty among Brazilian, Brazilian with Japanese genetic background and older Japanese women. To substantiate our

findings, we discussed our observations and results separately, detailing each KCL domain and linking it to the participants' sociodemographic and lifestyle characteristics.

According to the KCL domains, we observed differences regarding the mean scores in IADL ($P < 0.001$) and physical ($P = 0.047$) domains among the three groups; however, such differences failed to remain statistically significant when we dichotomized them according to the cut-off points to determine frailty. A similar pattern was observed in the nutritional domain; no group showed a significantly different risk level to develop frailty. Although no differences were found in the physical and nutritional domains among the groups, we can discuss the significant difference observed in BMI ($P < 0.001$), especially because BMI is an important indicator of physical and nutritional status, and an increased BMI could be an alarming sign of imminent frailty evaluated by both domains. In the present study, the Brazilian participants were more obese (BMI 28.1 ± 5.39 kg/m^2) than the other groups. Although the KCL considers low bodyweight to be a frailty symptom, epidemiological studies show that both overweight and underweight are negative health outcomes associated with a greater risk for morbidity and mortality.[15]

There are some data showing that the Brazilian environment might pose a risk for developing obesity compared with the Japanese environment; a study verified that the risk for developing central obesity was 2.8-fold higher among Japanese Brazilians living in Brazil.[8] Although that study did not include Brazilian natives, there is evidence supporting concurrent increases in obesity in Brazil.[16]

Furthermore, we found that Brazilian participants were threefold more likely to be frail in terms of oral health (eating domain) than the Japanese group (OR 3.18, 95% CI 1.47–6.85, $P = 0.003$). In this case, the educational level of the participants seems to be related to their poor oral condition; considering the evidence that older adults who received elementary school level education had a significantly larger number of missing teeth and significantly fewer healthy gingival units compared with those who received higher than elementary school level education.[17] Another study showed that not only educational level, but also living arrangement influenced the participants' oral health; concluding that poorly educated and divorced women had fewer remaining teeth than better-educated and married women.[18] In the present study, the most favored group in terms of educational and living arrangement conditions was the Japanese cohort that were also more concerned about dehydration (consuming liquids, especially tea, as one of the Japanese habits), another included aspect in the oral domain.

Regarding the socialization domain, the Brazilian participants also showed a greater risk for becoming frail compared with the Japanese participants (OR 9.15, 95% CI 3.53–23.7, $P < 0.001$). A study showed that a partner relationship, such as marriage, might impact women's health status in numerous ways and could confer health-related benefits, such as providing nurturing conditions and socialization through a spouse,[19] and building a network with the partner's family.[20] Furthermore, a relationship possibly includes access to material resources and other social support.[21] These privations could lead Brazilian women to a poorer condition not only in the seclusion domain, but also in the mood domain, as the study concluded that individuals who lack social connections or report frequent feelings of loneliness tend to suffer higher rates of depression as well.[22]

Although the older Brazilian women showed a higher life satisfaction ($P = 0.002$), they presented a higher risk for being frail in terms of depression (OR 6.63, 95% CI 2.74–16.0, $P < 0.001$) than the Japanese group. Evidence showed that living alone or with other(s) than a partner could lead to depression and anxiety disorders in women.[23]

Finally, the results that we found in the memory domain did not differ from those aforementioned. The Brazilian participants were threefold more likely to be frail compared with the Japanese group (OR 3.87; 95% CI 1.93–7.75, $P < 0.001$). It is widely recognized that low education is one of the conditions that affect cognitive performance, especially phonological verbal fluency, calculation and working memory[24,25] that are required when processing the tasks assessed by the KCL cognitive domain. Another factor that might be related to the lowest scores achieved by Brazilian women in the memory domain is their highest number of medication use (Brazilian participants 2.9 ± 2.1 *vs* Japanese participants 2.1 ± 1.5, $P = 0.028$). Although we did not investigate the drug classes, the cognitive impairment is repeatedly reported to be a side-effect among medications prescribed for the elderly.[26,27]

We discerned that the majority of the differences in the present study were shows between Japanese and Brazilian natives. However, we must emphasize that an improved condition in terms of frailty was observed in the Brazilian Japanese descendants. This result might be linked to their higher educational level that predicts a higher-level financial status and better living conditions, which might in turn reflect a better health education, as they showed the lowest total KCL score ($P < 0.001$), and also the lowest mean KCL score in physical strength ($P = 0.047$), eating ($P = 0.001$) and mood ($P < 0.001$) domains.

We emphasize that the native Brazilian participants might be more vulnerable and frail because of the sociodemographic disadvantages that they are exposed to and their adopted lifestyle. However, such conditions are reversible; and an early detection of the frail aspects

is essential to reverse it in older adults. For this purpose, the KCL was designed to monitor the health conditions and to detect negative health outcomes at the earliest stage, thereby assuring prompt prevention or rehabilitation interventions, being an accurate, cheap, easy and fast diagnostic tool.

The present study had several limitations: (i) the present study was a cross-sectional design, which did not enable us to determine a cause–effect relationship; (ii) the present study was carried out in only one Brazilian and one Japanese region, which did not allow us to extend our findings to the national level; and finally, (iii) we only analyzed older women with heterogeneous characteristics, which complicated our comparisons. We recommend prospective studies to include a greater sample size, with male participants recruited from several regions of Brazil and Japan, and that future studies investigate important aspects that could be related to frailty, such as the financial situation of the participants.

In summary, we found that Brazilian natives were more frail than Japanese natives, but not Brazilian Japanese descendants. In addition to the environment, we believe that the lifestyle and the sociodemographic conditions could reflect the frailty of older Brazilian women in the present study. Hence, we recommend the dissemination of general health education among these older adults, including incentives for regular engagement in physical activity and a well-balanced diet, the principles of oral health safety and social and cognitive approaches to warrant a healthy aging process.

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Disclosure statement

The authors declare no conflict of interest.

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SYSTEMATIC REVIEWS

Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS)

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Abstract

Objective: to examine the clinical evidence reporting the prevalence of sarcopenia and the effect of nutrition and exercise interventions from studies using the consensus definition of sarcopenia proposed by the European Working Group on Sarcopenia in Older People (EWGSOP).
Methods: PubMed and Dialog databases were searched (January 2000–October 2013) using pre-defined search terms. Prevalence studies and intervention studies investigating muscle mass plus strength or function outcome measures using the EWGSOP definition of sarcopenia, in well-defined populations of adults aged ≥50 years were selected.
Results: prevalence of sarcopenia was, with regional and age-related variations, 1–29% in community-dwelling populations, 14–33% in long-term care populations and 10% in the only acute hospital-care population examined. Moderate quality evidence suggests that exercise interventions improve muscle strength and physical performance. The results of nutrition interventions are

equivocal due to the low number of studies and heterogeneous study design. Essential amino acid (EAA) supplements, including ~2.5 g of leucine, and β-hydroxy β-methylbutyric acid (HMB) supplements, show some effects in improving muscle mass and function parameters. Protein supplements have not shown consistent benefits on muscle mass and function.
Conclusion: prevalence of sarcopenia is substantial in most geriatric settings. Well-designed, standardised studies evaluating exercise or nutrition interventions are needed before treatment guidelines can be developed. Physicians should screen for sarcopenia in both community and geriatric settings, with diagnosis based on muscle mass and function. Supervised resistance exercise is recommended for individuals with sarcopenia. EAA (with leucine) and HMB may improve muscle outcomes.

Keywords: *exercise intervention, nutrition intervention, prevalence, age-related, sarcopenia, older people*

Introduction

Although exercise and nutrition interventions have proved efficacy to treat different conditions in various populations of adults and older people, the effects in those with sarcopenia have received less attention. Sarcopenia has been defined as the loss of skeletal muscle mass and strength that occurs with advancing age [1]. However, until recently, there has been no widely accepted definition of sarcopenia that was suitable for use in research and clinical practice.

A practical clinical definition of, and consensus diagnostic criteria for, age-related sarcopenia was developed in 2009–10 and reported by the European Working Group on Sarcopenia in Older People (EWGSOP) [2]. The EWGSOP provided a working definition of sarcopenia as 'a syndrome characterised by progressive and generalised loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death' [2]. They proposed that sarcopenia is diagnosed using the criteria of low muscle mass and low muscle function (either low strength and/or low physical performance) [2]. A similar approach was taken in 2009 by the International Working Group on Sarcopenia (IWGS), who provided a consensus definition of sarcopenia as 'age-associated loss of skeletal muscle mass and function'. This group proposed that sarcopenia is diagnosed based on a low whole-body or appendicular fat-free mass in combination with poor physical functioning [3].

To date, most prevalence and intervention studies have used varied definitions of sarcopenia that are not current (e.g. based only on decreased muscle mass) and the results may therefore be misleading and difficult to interpret. However, with the implementation of new operational definitions of sarcopenia, it may be possible to define the natural course of the condition and determine which treatments are effective. In 2013, representatives of the EWGSOP, IWGS and international experts from Asia and America came together to form the International Sarcopenia Initiative (ISI) with the intention of developing a systematic review of some aspects of sarcopenia. Specifically, the aims of this systematic review were to (i) assess the prevalence of sarcopenia using definitions that include both muscle mass and muscle function, as proposed by the EWGSOP and the IWGS; and (ii) to review interventions with nutrition and exercise that used both muscle mass and muscle function as outcomes.

Methods

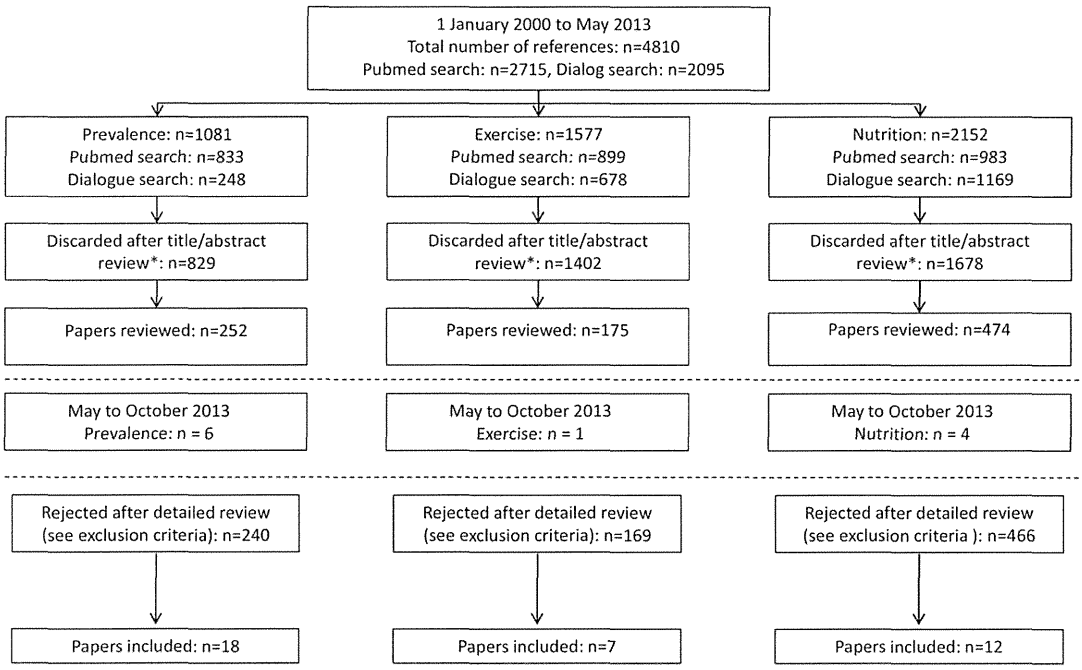
Search strategy

PubMed and Dialog databases were searched from January 2000 to May 2013 using the pre-defined search terms sarcopenia and muscle mass: additional pre-defined search terms were applied (see Supplementary data available in *Age and Ageing* online, Appendix S1) for each of the three areas of interest: prevalence of sarcopenia, nutrition interventions for sarcopenia and exercise interventions for sarcopenia (Figure 1). An additional short search of PubMed and Dialog databases using the terms 'sarcopenia', 'elderly', 'intervention', 'prevalence' and 'treatment' was conducted to cover articles published in the period May–October 2013 (Figure 1). The reference lists of systematic review articles and meta-analyses were scanned for any additional references missed from the PubMed and Dialog searches. The expert group was also asked to identify and provide any additional papers; they deemed to have been missed in the formal literature searches.

Eligibility criteria

Across all three categories, only studies that enrolled participants aged 50 years and older within well-defined populations (such as those in community-dwelling, hospital and nursing home/geriatric settings) were included. Prevalence studies were included if sarcopenia had been assessed according to the EWGSOP definition of sarcopenia, i.e. based on muscle mass *and* muscle strength *or* physical performance [2]. They were excluded if they only used muscle mass to define sarcopenia. Nutrition and exercise intervention studies were included if the outcome measures reported for the interventions included muscle mass and at least one measure of muscle strength or physical performance, even when the population studied was not defined as sarcopenic. If these outcomes were not clearly stated within the study methodology, the study was excluded. Other criteria used to exclude studies in each of the three categories are provided in Supplementary data available in *Age and Ageing* online, Appendix S2.

Observational studies were included in the prevalence category, but for the exercise and nutrition intervention categories, only randomised controlled trials were selected. The ISI group



*Papers discarded because they were duplicates or fell outside of the general topic or date range.

Figure 1. Selection of papers.

was divided into three subgroups (prevalence, exercise and nutrition). Final papers selected for inclusion in each of the three categories were agreed upon by each subgroup consensus.

Data synthesis

Data tables were compiled independently for each topic. For the prevalence of sarcopenia category, data were recorded on demographics (country, gender and age), assessment method used for each domain (muscle mass, muscle strength and physical performance) and sarcopenia prevalence. For the interventional categories, data were collected on population, numbers studied (by gender), age, intervention, control group, duration of intervention, outcomes measured and the main results. The methodological quality of each randomised, controlled trial was assessed using the 11-point Physiotherapy Evidence Database (PEDro) scale. Each item on the scale that the trial satisfied (except for item 1, which assesses external validity and is not included in the total score) contributed one point to the total PEDro score, with 0 representing the lowest score and 10 the highest [4]. This scale was specifically developed to rate the quality of randomised, controlled trials evaluating physical therapist interventions.

The following questions were investigated in patients aged 50 years and older without comorbid conditions. What is the prevalence of sarcopenia in different populations? Is physical exercise (as physical activity, resistance training or endurance training) effective compared with control in improving measures of muscle loss, muscle mass, muscle strength and physical performance? Compared with control, does nutrition supplementation improve measures of muscle mass, muscle strength, and physical performance? Based on the answers to these questions, draft recommendations were proposed by the co-chairs, and the working group then reviewed these recommendations to reach a consensus.

Results

Overall, 4810 publications were identified (Figure 1). Of these, 3909 were excluded, leaving 901 publications for potential inclusion (prevalence: 252; exercise: 175; nutrition: 474). In addition, 11 papers were identified as suitable for inclusion as a result of a short search of PubMed and Dialog databases to identify articles published in the period May–October 2013.

Eighteen prevalence, 7 exercise and 12 nutrition papers were finally chosen by the working group members for inclusion within this review (Figure 1).

Estimates of prevalence

Of the 18 prevalence studies meeting the inclusion criteria, 15 (83%) were in community-dwelling patients [5, **6–9**, 10, 11, **12, 13**, 14, 15, **16**, 17, **18**, 19], with two studies in patients in long-term care institutions [**20, 21**], and one publication in the acute hospital-care setting [**22**] (Table 1). The reporting of age varied across studies, but for those where the mean age was given, this ranged from 59.2 to 85.8 years [5, **6–9**, 10, 11, **12, 13**, 14, **16**, 17, **18**, 19, **21**].

Table 1. Prevalence of sarcopenia

Reference	Date data collected	Country	M/F, *n*	Assessment method			Age, years Mean (SD) [Range]	Sarcopenia prevalence, %		
				Muscle mass	Muscle strength	Physical performance		Total	Male	Female
Community-dwelling populations										
Abellan van Kan *et al.* [5]	Jan 1992–Jan 1994	France	0/3025	DEXA	HS	GS	80.51 (3.9) [≥75]	5.2	–	5.2
Landi *et al.* [6]	Oct 2003	Italy	66/131	MAMC	HS	GS	82.2 (1.4) [80–85]	21.8	25.7	19.8
Landi *et al.* [7]	Oct 2003	Italy	118/236	MAMC	HS	GS	85.8 (4.9)	29.1	27.1	30.1
Lee *et al.* [8]	–	Taiwan	223/163	DXA	HS, KE, PEF	SPPB, GS, TUG, or SCPT	73.7 (5.6)	7.8[a] 16.6[b]	10.8[a] 14.9[b]	3.7[a] 19.0[b]
Legrand *et al.* [9]	Nov 2008–Sep 2009	Belgium	103/185	BIA	HS	mSPPB, GS	84.8 (3.6) [>80]	12.5	14.6	12.4
Malmstrom *et al.* [10]	Sep 2000–Jul 2001	USA (African Americans)	124/195	DEXA	–	GS	59.2 (4.4)	4.1	–	–
McIntosh *et al.* [11]	–	Canada	42/43	BIA	HS	GS	75.2 (5.7)	6.0	S: 5 SS: 0	S: 7 SS: 0
Murphy *et al.* [12]	–	USA	1426/1502	DEXA	HS	GS	F: 73.5 (2.88) M: 73.8 (2.85) Total: [70–79]	S: 5	–	–
Patel *et al.* [13]	–	UK[c]	Cohort A: 103/0 Cohort B: 765/1022	DEXA, SFT	HS	GS, TUG, chair-rise time	(A): 72.5 (2.5) (B): M, 67.0 (2.6); F, 67.1 (2.6)	(A): 6.8 (B): 7.8	4.6	7.9
Patil *et al.* [14]	–	Finland	0/409	DEXA	HS	GS, SPPB, TUG	74.2 (3.0) [70–80]	0.9	–	0.9
Sanada *et al.* [15]	–	Japan	0/533	DEXA	HS, LEP	Sit and reach, VO_{2max}	<39: 11.4% <49: 21.2% <59: 25.9% <69: 29.8% <85: 11.6% [30–84]	24.2	–	24.2
Tanimoto *et al.* [16]	May–Jun 2007, 2008, 2009	Japan	364/794	BIA	HS	GS	M: 74.4 (6.4) F: 73.9 (6.3) [≥65]	–	11.3	10.7
Verschueren *et al.* [17]	–	Belgium, UK	679/0	DEXA	HS, KE	GS	59.6 (10.7) [40–79]	S: 3.7 SS: 0	–	–
Volpato *et al.* [18]	2004–2006	Italy	250/288	BIA	HS	GS	77.1 (5.5) [65–97]	10.2	2.6	6.7
Yamada *et al.* [19]	–	Japan	568/1314	BIA	HS	GS	74.9 (5.5) [65–89]	–	21.8	22.1
Institutional dwelling										

Continued

Table 1. Continued

Reference	Date data collected	Country	M/F, *n*	Assessment method			Age, years Mean (SD) [Range]	Sarcopenia prevalence, %		
				Muscle mass	Muscle strength	Physical performance		Total	Male	Female
Bastiaanse *et al.* [20]	–	Netherlands	450/434	CC	HS	GS	50–59: 46.5% 60–69: 35.2% 70–79: 16.2% ≥80: 2.1% [≥50]	All: 14.3 50–64: 12.7 ≥65: 17.4	–	–
Landi *et al.* [21]	Aug–Sep 2010	Italy	31/91	BIA	HS	GS	84.1 (4.8) [≥70]	32.8	67.7	20.8*
Acute hospital care Gariballa and Alessa [22]	–	UK	227/205	MAMC	HS	–	[≥65]	10.2	–	–

ALM, appendicular lean mass; BIA, bioelectrical impedance analysis; CC, calf circumference; DEXA, dual-energy X-ray absorptiometry; F, female; GS, gait speed; HS, hand-grip strength using a dynamometer; KE, knee extensor; LEP, leg extension power; M, male; MAMC, mid-arm muscle circumference; PEF, peak expiratory flow; S, sarcopenia; SCPT, stair-climb power test; SD, standard deviation; SFT, skin-fold thickness; (m)SPPB, (modified) standard physical performance battery; SS, severe sarcopenia; TUG, timed-up-and-go; VO_{2max}, maximal oxygen uptake.

[a]By relative appendicular skeletal muscle index.

[b]By percentage skeletal muscle index.

[c]Consists of two cohorts (Cohort A: detailed data were collected. Cohort B: same data were collected, but no DEXA).

*$P < 0.001$ versus females.

The prevalence of EWGSOP-defined sarcopenia was 1–29% (up to 30% in women) for older adults living in the community [5, **6–9**, 10, 11, **12, 13**, 14, 15, **16**, 17, **18**, 19], 14–33% (up to 68% in men) for those living in long-term care institutions [**20, 21**] and 10% for those in acute hospital care [**22**]. Age was not consistently reported across the studies, with some giving mean ages only, others reporting ranges, and others breaking age down into categories; thus, a comprehensive analysis of prevalence based on age could not be made. However, where reported, the majority of studies suggested the prevalence of sarcopenia increased with age [**18**, 19, **22**]. However, one study appeared to show a decrease in sarcopenia prevalence with increasing age [**20**]. In one study, sarcopenia appeared to be related to gender, with males more commonly affected than females [**21**], while another study showed a numerically higher prevalence of sarcopenia and severe sarcopenia in women than in men [**13**]. In a further study, the prevalence of sarcopenia was higher in women than in men in those aged <75 years; but, in those aged >85 years, the prevalence of sarcopenia was higher in men than in women ($P < 0.05$) [19]. However, in most studies that reported gender, there was no significant association with sarcopenia prevalence [**6–9**, 11, **16**, 19, **20**].

Exercise interventions

There were seven moderate quality (PEDro score: 4–6) intervention studies that investigated the effect of exercise on muscle parameters in different populations aged 60–95 years (Table 2) [**23–29**]. The impact of exercise on sarcopenia was assessed using muscle mass and muscle strength or power measures in all studies [**23–29**]; assessment of physical performance (chair rise [**24**], 12-min walk [**25**], stair climbing [**29**] or timed up and go [**27, 28**]) was carried out in five of seven studies (Table 2).

Resistance training interventions

Resistance training was explored in four mixed-gender studies (Table 2) [**23–25, 29**]. When used from 3–18 months, resistance training interventions alone improved muscle mass in two of four studies [**23, 29**] and muscle strength in three of four studies [**23, 25, 29**] compared with control (low-intensity home exercise or standard rehabilitation). Physical performance (chair rise, stair climb or 12-min walk) improved with resistance training alone versus control in all three studies assessing this parameter [**24, 25, 29**].

Combined exercise/physical activity interventions

Three additional studies explored compound exercise interventions (with different blends of aerobic, resistance, flexibility and/or balance training), which were performed for 3–18 months [**26–28**]. A high-intensity multipurpose exercise programme over 18 months improved muscle mass, muscle strength and physical performance versus control (wellbeing) in a study in 246 women [**27**]. In two mixed-gender studies

Table 2. Summary of the effect of exercise on sarcopenia in randomised, controlled studies meeting the inclusion criteria

Reference	Population	Number studied (M/F)	Age, years Mean (SD) [Range]	Intervention		PEDro score	Outcomes measured	Main results
				Description	Duration (months)			
Binder *et al.* [23]	Frail, community-dwelling	91	83 (4)	Progressive RET; CON (low-intensity home exercise)	9	5	MM (DEXA), MS (KE)	Total body FFM increased in the progressive RET group, but not in the CON group ($P = 0.005$) MS increased to a greater extent in the progressive RET than in the CON group ($P = 0.05$)
Bonnefoy *et al.* [24]	Frail, care institution	57 (7/50)	83	RET + SUPP; CON + SUPP; RET + PLA; PLA + CON	9	5	MM (FFM by labelled water), MP, PP (chair rise)	RET did not improve MM or MP, but improved PP versus CON ($P = 0.01$)
Bunout *et al.* [25]	Community-dwelling	98 (36/62)	≥70	RET + SUPP; SUPP; RET; CON	18	4	MM (DEXA), MS (quadriceps strength), PP (12-min walk)	FFM did not change in any group RET improved MS versus CON ($P < 0.01$) PP remained constant in RET group, but declined in the CON group ($P < 0.01$).
Suetta *et al.* [29]	Frail, post-operative elective hip replacement	36 (18/18)	[60–86]	RET; ES; CON (standard rehabilitation)	3	5	MM (US), MS (quadriceps), PP (stair climbing)	RET improved MM, MS and PP versus CON (all $P < 0.05$) In the ES or CON groups, there was no increase in any measurement outcomes
Goodpaster *et al.* [26]	Sedentary, community-dwelling	42 (11/31)	[70–89]	PA (aerobic, strength, flexibility, balance training); CON (health education)	12	5	MM (CT scan), MS (KE)	MM decreased in both groups (but losses were not different between groups) MS loss was decreased in CON, but completely prevented in PA (between group change not significant)
Kemmler *et al.* [27]	Community-dwelling	246 (0/246)	69.1 [65–80]	High-intensity multipurpose exercise programme; CON (wellbeing)	18	6	MM (DEXA), MS (isometric leg extension), PP (timed up and go)	Multipurpose exercise was associated with significant improvements in MM ($P = 0.008$), MS ($P = 0.001$), PP ($P < 0.001$) versus CON
Rydwik *et al.* [28]	Frail, community-dwelling	96 (38/58)	>75	PA (aerobic, muscle strength, balance exercises); nutrition intervention; PA + nutrition intervention; CON	3	5	MM [FFM = BW-fat mass (skin folds)], MS (leg press, dips), PP (timed up and go)	PA improved MS ($P < 0.01$ for dips), but did not improve MM or PP versus CON

BW, body weight; CON, control; CT, computerised tomography; DEXA, dual-energy X-ray absorptiometry; ES, electrical stimulation; F, female; FFM, free-fat mass; FM, fat mass; KE, knee extension; M, male; min, minute; MM, muscle mass; MP, muscle power; MS, muscle strength; RET, resistance exercise training; PA, physical activity; PLA, placebo; PP, physical performance; SD, standard deviation; SUPP, nutritional supplement; US, ultrasound.

[26, 28], muscle mass did not improve; muscle strength (assessed as dips) improved with physical activity versus control at 3-months follow-up in one of the two studies [28]; and physical performance did not improve in the one study in which it was assessed [28].

Overall, most exercise trials showed improved muscle strength and physical performance (using different measures), but only three of seven studies found increased muscle mass. These trials were largely performed in community-dwelling older people, sometimes identified as frail by different measures.

Nutrition interventions

Most studies (11/12) evaluating nutrition intervention in adults aged 50 years and over (Table 3) were in community-dwelling populations whose age ranged from 62 to 90 years (n = 14–98) [25, 30–39]. One study assessed individuals living in care institutions (mean age, 83 years; n = 57) [24]. Nutrition interventions that were identified included protein supplementation (usually with other nutrients providing extra calories) [24, 25, 30, 37, 38], amino acid (mainly leucine) supplementation [33, 35], β-hydroxy β-methylbutyric acid (HMB; a bioactive metabolite of leucine) supplementation with arginine [34] or alone [32, 34, 36, 39] or fatty acid supplementation [31] administered over 8–36 weeks to evaluate changes in muscle mass and/or strength and function.

Protein supplements

Protein supplementation (with other nutrients providing ~400 extra kilocalories/day in three of five studies) either alone or in addition to resistance exercise training was evaluated in five moderate- to high-quality (PEDro score: 4–10) studies [24, 25, 30, 37, 38]. In the only high-quality study with no associated exercise in a frail, community-dwelling population, protein supplementation improved physical performance, but not muscle mass or muscle strength versus control [38]. Only in one of the four moderate- to high-quality studies using different types and amounts of protein supplementation in addition to an exercise programme for 24 weeks to 18 months [24, 25, 30, 37], was muscle mass increased over the control group [40]. Muscle strength did not change in any of the studies; only a transient increase in muscle power was found in one study [24]. Physical performance did not improve in any of these four studies.

Overall, these five moderate- to high-quality studies fail to show a consistent effect of protein supplementation on muscle mass and function [24, 25, 30, 37, 38].

Essential amino acid supplementation

The effect of essential amino acid (EAA) supplementation either alone [33] or in combination with resistance exercise training [35] on muscle parameters was investigated in two high-quality (PEDro score: 7 and 8) studies of 3 month's duration each, in community-dwelling individuals. Daily leucine amount provided was 2.8 and 2.5 g. EAA improved muscle mass in one of two studies [33], did not improve muscle strength, and improved physical performance in the study that used this outcome [35]. When combined with exercise, EAA improved leg muscle mass and muscle strength but not physical performance versus health education at 3 months [35].

Overall, very limited evidence on EAA supplementation seems to show some effects on muscle mass and function.

HMB supplementation

The effect of HMB alone [32, 36] or HMB in combination with ARG and LYS [34] or resistance exercise training [39] on muscle parameters has been investigated in four high-quality (PEDro score: 8–10) studies of 8–24-week duration in community-dwelling older adults [34, 36, 39] or in healthy older adults on extended bed rest [32]. HMB prevented muscle mass loss in one of four studies and did not improve muscle mass in the other three [32]; improved muscle strength in one [34] (and possibly two) [36] of four studies and improved physical performance in one of four studies [34].

Overall, HMB showed some effects on muscle mass and function in these high-quality studies, but sample sizes were small.

Fatty acids

The only study examining the effect of fatty acid supplementation (α-linolenic acid) on muscle parameters (PEDro score: 10), in 51 older adults undergoing resistance training for 12 weeks, showed no effect of the supplementation on muscle mass or muscle strength versus placebo [31].

Discussion

Sarcopenia is an independent risk factor for adverse outcomes, including difficulties in instrumental and basic ADL [6, 10, 16, 20, 21], osteoporosis [17], falls [21], hospital length of stay and re-admission [22] and death [6]. This underscores the importance of understanding the true prevalence of sarcopenia and effective preventative strategies.

Prevalence

The prevalence of sarcopenia in the literature varies widely, and is likely to be affected by the population studied (including the population under investigation and the reference population) and the different methods used to assess muscle mass, muscle strength and physical performance [3]; although results may also be due to real differences in prevalence of sarcopenia. As studies that defined sarcopenia as muscle mass plus muscle strength/physical performance were few, comparisons of prevalence across studies were difficult due to the different methods and cut-off points

Table 3. Summary of the effect of nutrition on sarcopenia in randomised, controlled studies meeting the inclusion criteria

Reference	Population	Number studied (M/F)	Age, years, mean (SD) [range]	PEDro Score	Intervention (duration)	Outcomes measured	Main results
Bonnefoy *et al.* [24]	Frail, care institution	57 (7/50)	83	5	RET + SUPP (400 kcal, 30 g of protein/day); CON + SUPP; RET + PLA; PLA + CON (9 months)	MM (FFM by labelled water), MP, PP (chair rise, 6-min walk, stair climb)	SUPP significantly increased MP at 3 months versus CON ($P = 0.03$), but not at 9 months SUPP did not improve MM or PP versus CON
Bunout *et al.* [25]	Community-dwelling	98 (36/62)	[≥70]	4	RET + SUPP (400 kcal, 13 g of protein/day); SUPP; RET; CON (18 months)	MM (DEXA), MS (biceps and quadriceps strength), PP (12-min walk)	SUPP alone had no effect on MM, MS or PP SUPP did not show an additive effect over RET outcome
Chale *et al.* [30]	Sedentary, community-dwelling	80 (33/47)	[70–85]	10	WPS (378 kcal, 40 g of protein/day) + RET; CON (378 kcal, no protein) + RET (6 months)	MM (DEXA, CT scan), MS (KE), PPPP (stair climb, chair rise, 400 m walk, SPPB)	WPS + RET did not improve MM, MS or PP significantly versus CON + RET
Tieland *et al.* [37]	Frail, community-dwelling	62 (21/41)	PLA: 79 (6) Protein: 78 (9) [≥65]	10	Protein (30 g/day) + RET; PLA + RET (24 weeks)	MM (DEXA), MS (leg press, LE, HS), PP (SPPB)	Protein + RET significantly improved MM ($P = 0.006$), but not MS or PP versus PLA + RET
Tieland *et al.* [38]	Frail, community-dwelling	65 (29/36)	PLA: 81 (±1 SEM) Protein 78 (±1 SEM) ≥65	8	Protein (30 g/day); PLA; (24 weeks)	MM (DEXA), MS (leg press, LE, HS), PP (SPPB)	PP improved significantly with protein supplementation ($P = 0.02$), but not MM or MS versus PLA
Dillon *et al.* [33]	Healthy individuals	14 (0/14)	All: 68 (±2) PLA: 69 (±3) Supplement: 67 (±1)	7	EAA (HIS, ILE, LEU, LYS, MET, PHE, THR, VAL); PLA; (3 months)	MM (DEXA), MS (bicep curl, triceps extension, LE, leg curl)	EAA increased MM versus baseline, ($P < 0.05$) There were no changes in MS
Kim *et al.* [35]	Community-dwelling	155 (0/155)	79 (2.9) [≥75]	8	EAA (LEU, LYS, VAL, ILE, THR, PHE) + RET; EAA; RET; HE (3 months)	MM (BIA), MS (KE), PP (max. walking speed)	EAA alone improved PP, but not MM and MS versus HE EAA + RET improved leg (not appendicular or total) MM ($P < 0.007$) and, MS ($P = 0.02$) versus HE PP was not more improved by the addition of EAA than by RET alone
Flakoll *et al.* [34]	Community-dwelling	57 (0/57)	76.7 [62–90]	8	ARG + HMB + LYS; PLA (12 weeks)	MM (BIA), MS (isometric leg strength, HS), PP (get up and go)	MS ($P \leq 0.05$) and PP ($P = 0.002$) significantly improved with ARG + HMB + LYS versus PLA ARG + HMB + LYS did not significantly improve MM versus PLA
Deutz *et al.* [32]	Healthy individuals on bed rest	19 (4/15)	PLA: 67.1 (±1.7) HMB: 67.4 (±1.4) [60–76]	10	HMB; PLA Bed rest (10 days) + rehabilitation (8 weeks)	MM (DEXA), MS (KE, leg press), PP (SPPB, get up and go, 5-item PPB)	Bed rest caused a significant decrease in MM ($P = 0.02$) in the PLA group, but MM was preserved in the HMB group Changes in MS and PP were not significant for HMB versus PLA

Continued

Table 3. Continued

Reference	Population	Number studied (M/F)	Age, years, mean (SD) [range]	PEDro Score	Intervention (duration)	Outcomes measured	Main results
Stout *et al.* [36]	Community-dwelling	98 (49/49)	73 (±1 SEM) [≥65]	9	Phase I: HMB; PLA (24 weeks) Phase II: PLA + RET; HMB + RET (24 weeks)	MM (DEXA), MS (isokinetic leg strength, HS), PP (get up and go)	HMB alone significantly improved some, but not all measures of MS versus PLA. No significant changes were found in MM and PP with HMB versus PLA Adding HMB to RET did not improve any parameters over RET alone
Vukovich *et al.* [39]	Community-dwelling	31 (15/16)	70 (±1)	10	HMB + RET; PLA + RET (8 weeks)	MM (DEXA, CT scan), MS (misc. upper and lower body strength press, flexion and extension measurements)	MM improved with HMB + RET versus PLA + RET, but not significantly ($P = 0.08$) MS did not improve with HMB + RET versus PLA + RET
Cornish and Chilibeck [31]	Community-dwelling	51 (28/23)	65.4 (±0.8)	10	ALA + RET; PLA + RET (12 weeks)	MM (DEXA, US), MS (leg press, chest press)	ALA + RET had minimal effect on MM or MS versus PLA + RET

ALA, α-linolenic acid; ARG, arginine; BIA, bioelectrical impedance analysis; CON, controls; CT, computerised tomography; DEXA, dual X-ray absorptiometry; EAA, essential amino acid; F, female; FFM, fat-free mass; HE, health education; HIS, histidine; HMB, β-hydroxy β-methylbutyrate; ILE, isoleucine; HS, hand-grip strength; KE, knee extension; LE, leg extension; LEU, leucine; LYS, lysine; M, male; min, minute; MET, methionine; MM, muscle mass; MP, muscle power; MS, muscle strength; NS, not significant; PHE, phenylalanine; PLA, placebo; PP, physical performance; RET, resistance exercise training; SD, standard deviation; SPPB, standard physical performance battery; SUPP, nutritional supplement; THR, threonine; VAL, valine; WPS, whey protein supplement.

used. The prevalence of sarcopenia in the community using a definition consistent with EWGSOP was 1–33% across different populations (male and female data combined), with higher prevalence, as expected, in settings where older, more complex or acutely ill individuals are cared for. Ethnicity may also play a role, especially if the reference and study populations do not match.

After careful consideration of the methodological limitations and scope of these studies, the ISI group proposes certain recommendations for the design of future studies (expert advice):

- Studies with sufficient sample size to identify prevalence and risk factors for sarcopenia, including subpopulation analyses, are needed.
- Studies should focus on standardised, well-defined, reproducible populations, namely community-dwelling individuals, individuals living in nursing homes/care homes, and acutely ill or physically frail inpatients. These populations should be clearly described so that studies can be compared for external validity.
- Standardised models and cut-off points should be used for each domain of the definition of sarcopenia to allow comparison between studies.
- Longitudinal studies on the incidence of sarcopenia are needed, again using standard methods.

Exercise intervention

Exercise interventions appear to have a role in increasing muscle strength and improving physical performance, although they do not seem to consistently increase muscle mass, in frail, sedentary, community-dwelling older individuals. Investigations in other populations are still anecdotal. No trials were found that recruited individuals based on their sarcopenic status. The results suggested that combining various types of exercise into a programme may also improve muscle strength and physical performance. Most exercise studies involved limited participants and were mainly conducted within a single country.

Recommendations for the design of exercise studies (expert advice):

- Improved standardisation of exercise interventions is needed, to allow for replication and contrast.
- Studies should have common outcome measures, along with similar time points for assessment (e.g. 4 weeks, 8 weeks, 3 months, 6 months, 1 year), so that valid comparisons across studies can be made. The short physical performance battery, gait speed, 400-m walking distance and grip strength are proposed as useful measures of physical performance that are able to determine clinically significant changes. Grip strength, chair rise and knee extension may be used to measure muscle strength.
- Exercise interventions should focus on well-defined populations, with well-defined sarcopenia.

Nutrition intervention

Although nutrition intervention is considered one of the mainstays of intervention in sarcopenia, much of the evidence is based on short-term protein synthesis studies, and large clinical trials are still lacking. Our review has failed to show a consistent effect of protein supplementation, although the number of studies found using our strict selection criteria was very low. EAAs (with ~2.5 g of leucine) and HMB seem to have some effects on muscle mass and muscle function that need to be confirmed in larger trials. Vitamin D studies were evaluated as part of the review process; while some epidemiological studies link vitamin D levels with muscle parameters, there were no intervention studies meeting the criteria for inclusion in this review. Similarly, there is a large literature on the effects of omega 3-fatty acids on muscle parameters, especially in cachexia, but only one negative study was found in this review [**31**]. Interventions that evaluated the combined effects of exercise and nutrition sometimes suggested a potential additive effect, although this needs further research. However, solid evidence on which to base recommendations for patients with sarcopenia is not available.

Recommendations for the design of nutrition studies (expert advice)

- Further studies are needed to determine the effect of different nutrition interventions on muscle mass and function using robust, multi-centre and standardised approaches with single or complex nutrition interventions and clinically relevant outcomes (muscle strength, physical performance).
- Studies using four arms (exercise, nutrition, both or none) should also be conducted. The choice of exercise and nutrition interventions should be based on the singular effect of each intervention.
- Outcome measures for such studies should not differ from those used for individual components, and reporting should allow for individual group comparisons to also evaluate the role of each component.
- Timing of nutrition intervention before or after exercise should be explored in clinical trials comparing different times of administration, as basic studies suggest there may be time-associated differences in the effect of nutrition intervention over exercise.
- Baseline nutritional status and physical frailty of the population should be considered when doing nutrition intervention studies.

Practice recommendations

Sarcopenia is a common clinical problem in people over 50 years of age, and one that leads to severe adverse outcomes. Research on management interventions is advancing quickly, but questions still remain. Based on our current understanding, the expert group agreed some general recommendations for clinical practice (expert opinion):

(1) Sarcopenia, defined as low muscle mass and low muscle function and/or reduced physical performance, occurs in at least 1 in 20 community-dwelling individuals, and prevalence may be as high as 1 in 3 in frail older people living in nursing homes (Table 1).
   - Owing to the consequences of sarcopenia on quality of life, disability and mortality, it is recommended that physicians should consider screening for sarcopenia, both in community and geriatric settings.
   - The new definitions of sarcopenia, based on muscle mass and function, should be preferred to definitions based on muscle mass alone.

(2) Exercise interventions, especially those based on resistance training, may have a role in improving muscle strength and physical performance (moderate quality evidence), but not muscle mass. Moreover, exercise has been shown to improve other common conditions in adults and older patients, as well as being safe.
   - Supervised resistance exercise or composite exercise programmes may be recommended for frail or sedentary community-dwelling individuals.
   - Time of intervention of at least 3 months and probably longer may be needed to obtain significant improvement in relevant clinical parameters (muscle strength and physical performance). Increased physical activity in daily life may also be recommended in these individuals.

(3) Some nutrition interventions such as EAAs (with ~2.5 g of leucine) and HMB may improve muscle parameters. Although our findings did not appear to support this approach, increasing protein intake to 1.2 g/kg body weight/day, either by improving diet or adding protein supplements, has been recommended for adults and older people by an expert group [**40**]. Evidence to recommend specific interventions is yet to be established.

Key points

- The reported prevalence of sarcopenia in the community is up to 33%, with higher prevalence in long-term and acute care settings.
- This underscores the importance of preventative and clinical management strategies for managing sarcopenia.
- While further research is needed on interventions, we provide recommendations for clinical practice.
- The ISI included representatives of the European Working Group on Sarcopenia in Older People (EWGSOP), the International Working Group on Sarcopenia (IWGS) and international experts.

Conflicts of interest

Abbott had no role in the choice of members of the group, but had the right to have an observer member at the meetings. Members of the Working Group received no salary or other incomes from the European Union Geriatric medicine Society (EUGMS), Abbott Nutrition (AN) or any other

organisation for any of the tasks involved in the preparation of this manuscript or for attending the meetings of the group. An individual COI form has been filled by each member of the International Sarcopenia Initiative group. Medical writing support was provided by Mike Musialowski at Lucid with funding from AN.

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Supplementary data

Supplementary data mentioned in the text is available to subscribers in *Age and Ageing* online.

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The full list of references is available on Supplementary data available in *Age and Ageing* online, Appendix S3.

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A systematic review of outcomes following emergency transfer to hospital for residents of aged care facilities

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Abstract

Background: residential aged care facility (RACF) resident numbers are increasing. Residents are frequently frail with substantial co-morbidity, functional and cognitive impairment with high susceptibility to acute illness. Despite living in facilities staffed by health professionals, a considerable proportion of residents are transferred to hospital for management of acute deteriorations in health. This model of emergency care may have unintended consequences for patients and the healthcare system. This review describes available evidence about the consequences of transfers from RACF to hospital.
Methods: a comprehensive search of the peer-reviewed literature using four electronic databases. Inclusion criteria were participants lived in nursing homes, care homes or long-term care, aged at least 65 years, and studies reported outcomes of acute ED transfer or hospital admission. Findings were synthesized and key factors identified.
Results: residents of RACF frequently presented severely unwell with multi-system disease. In-hospital complications included pressure ulcers and delirium, in 19 and 38% of residents, respectively; and up to 80% experienced potentially invasive interventions. Despite specialist emergency care, mortality was high with up to 34% dying in hospital. Furthermore, there was extensive use of healthcare resources with large proportions of residents undergoing emergency ambulance transport (up to 95%), and inpatient admission (up to 81%).
Conclusions: acute emergency department (ED) transfer is a considerable burden for residents of RACF. From available evidence, it is not clear if benefits of in-hospital emergency care outweigh potential adverse complications of transfer. Future research is needed to better understand patient-centred outcomes of transfer and to explore alternative models of emergency healthcare.

Keywords: *emergency, nursing homes, older people*

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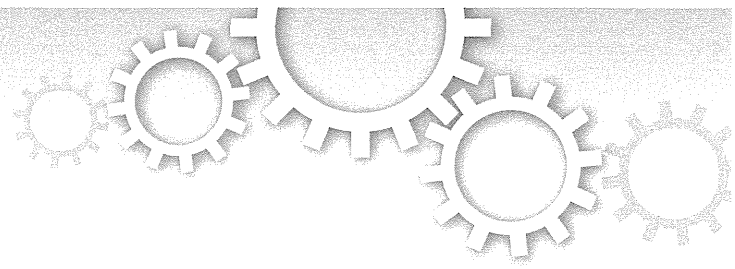

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AMAP1 as a negative-feedback regulator of nuclear factor-κB under inflammatory conditions

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NF-κB is a major transcriptional factor regulating many cellular functions including inflammation; therefore, its appropriate control is of high importance. The detailed mechanism of its activation has been well characterized, but that of negative regulation is poorly understood. In this study, we showed AMAP1, an Arf-GTPase activating protein, as a negative feedback regulator for NF-κB by binding with IKKβ, an essential kinase in NF-κB signaling. Proteomics analysis identified AMAP1 as a binding protein with IKKβ. Overexpression of AMAP1 suppressed NF-κB activity by interfering the binding of IKKβ and NEMO, and deletion of AMAP1 augmented NF-κB activity. The activation of NF-κB induced translocation of AMAP1 to cytoplasm from cell membrane and nucleus, which resulted in augmented interaction of AMAP1 and IKKβ. These results demonstrated a novel role of AMAP1 as a negative feedback regulator of NF-κB, and presented it as a possible target for anti-inflammatory treatments.

Nuclear Factor-κB (NF-κB) consists of a family of transcription factors (p65 or RelA, p50, p52, c-Rel, and RelB) that play critical roles in inflammation, immunity, cell proliferation, differentiation and survival[1]. It generally exists as a homo- or heterodimer in the cytosol that is bound to the inhibitor of κB (IκB) (see review[2]). In response to a wide variety of stimuli, including inflammatory cytokines, IκB is phosphorylated and degraded via the ubiquitin pathway, which is followed by NF-κB translocation to the nucleus and activation of transcription. Serine phosphorylation of IκB is mediated by a large multi-unit complex containing two catalytic subunits (IKKα and IKKβ) and the regulatory subunit IKKγ or NEMO[3,4]. Among these subunits, IKKβ is the essential kinase mediating IκB phosphorylation in most cell types, and germline deletion of IKKβ results in embryonic lethality due to massive liver apoptosis, similar to the phenotype of p65-knockout mice[5].

NF-κB signaling proteins including these kinases are essential for maintaining living body as shown by IKKβ or p65 knockout mouse with phenotype of embryonic lethality, but they also have been implicated in the pathogenesis of many human diseases, including cancer[6–9]. Therefore, controlling their activities appropriately is of high importance. Although the mechanism for its activation was been well analyzed as described above, that for negative regulation is poorly explored.

We recently reported that IKKβ has a kinase-independent role in regulating widespread cellular responses and cell signaling[10], implicating that there are still undiscovered roles of IKKβ. To explore the roles more extensively, we identified A Multiple-domain Arf-GAP Protein 1 (AMAP1) as a binding partner of IKKβ by proteomic analyses. AMAP1, also called ASAP1 or DDEF1, is an Arf-GTPase-activating protein that functions on membrane surfaces to catalyze the hydrolysis of GTP bound to Arf, and plays major roles in the regulation of membrane remodeling and cytoskeletal organization, cellular migration and tumor invasion and metastasis[11–14]. More importantly, clinical studies have indicated that AMAP1 is dramatically up-regulated in advanced cancers[15–22]. The roles of AMAP1 in the inflammatory responses are of high interest considering the close relationship between caner and inflammation[2]; however, the roles have been poorly understood. Interestingly, Haque et al.

reported the inhibitory effect of AMAP1 (ASAP1) on the production of proinflammatory mediators[23], but the detailed mechanism remains unknown.

In this report, we carefully examined the involvement of AMAP1 in the regulation of NF-κB, and found that it has an unanticipated role to interfere the IKKβ−NEMO binding, which results in negative-feedback regulation of NF-κB activity. This report describes the detailed role of AMAP1 in the inflammatory responses, and AMAP1 can be a new target for developing anti-inflammatory treatments.

Results

AMAP1 interacts with IKKβ. To discover novel roles of IKKβ, we started with identifying proteins that bind to IKKβ. Three kinds of FLAG-tagged IKKβ were overexpressed in HEK293T cells, and binding proteins were immunoprecipitated using anti-FLAG antibody. The samples were subjected to proteomic analyses using the nano-LC/MALDI-TOF system. Of the proteins identified repeatedly, we focused on AMAP1 since it is clinically important due to its augmented expression in cancer[15,16,19,22,24], which is one of the major inflammatory diseases[7,9]. To confirm the physical association of IKKβ and AMAP1, we performed co-immunoprecipitation (Co-IP) assays using the lysate of HEK293T cells co-overexpressed with HA-tagged AMAP1 and FLAG-tagged IKKβ. IKKβ and AMAP1 were detected in the precipitates with either the anti-HA antibody or the anti-FLAG antibody, respectively (Fig. 1a).

Next, we determined the binding sites of these proteins. Based on the functional modules of IKKβ, including an amino-terminal kinase domain (KD), a ubiquitin-like domain (ULD), an elongated α-helical scaffold/dimerization domain (SDD) and a carboxyl-terminal NEMO Binding Domain (NBD)[25], we divided IKKβ into 4 overlapping FLAG-tagged fragments. The constructs are IKKβ-Δ1 comprising the KD, ULD and SDD, IKKβ-Δ2 comprising the KD and ULD, IKKβ-Δ3 comprising only the KD, and IKKβ-Δ4 comprising the ULD, SDD and NBD (Fig. 1b), and each construct was expressed in HEK293T cells along with HA-tagged AMAP1. We found that AMAP1 was co-precipitated only with IKKβ-Δ4, but not with any other construct of IKKβ (Fig. 1c), indicating that AMAP1 binds to IKKβ at the C-terminal region containing the NBD. Further, we examined which region of AMAP1 is essential for its binding to IKKβ. Each of 6 GST-tagged fragments of AMAP1, which are Bar (Bin-Amphiphysin-Rvs), PH (Pleckstrin Homology), Arf-GAP, ANK (Ankyrin repeat), PRD (Proline Rich Domain) and SH3 (Src Homology3) domains (Fig. 1d), was expressed in HEK293T cells along with FLAG-tagged IKKβ. We found only SH3 domain construct was co-precipitated with IKKβ as shown in Fig. 1e. To confirm it further, we made construct of SH3-domain deleted AMAP1 (AMAP1ΔSH3). Cells were expressed with full-length AMAP1 or AMAP1ΔSH3 along with FLAG-tagged IKKβ, and we found only full-length AMAP1, not AMAP1ΔSH3, was co-precipitated with IKKβ (Supplementary Fig. S1 online). Taken together, these results indicated that the SH3 domain of AMAP1 is responsible for the binding with IKKβ, which implicates that the GTPase activity of AMAP1 is not involved in the interaction with IKKβ.

AMAP1 negatively regulates NF-κB. The C-terminal region of IKKβ identified as the binding site for AMAP1 contains the binding domain for NEMO/IKKγ, which is the regulatory subunit of the IKK complex[3]. It prompted us to verify the effect of AMAP1 on the association of IKKβ and NEMO because it is required for NF-κB activation[26]. As shown in Fig. 2a, overexpression of AMAP1 inhibited the binding of IKKβ and NEMO, and it was in a dose-dependent manner (Supplementary Fig. S2 online). In addition, the increased level of NEMO could mitigate the interaction between AMAP1 and IKKβ (Fig. 2b). Taken together, there is competition between AMAP1 and NEMO in IKKβ interaction. As expected from these results, overexpression of AMAP1 suppressed NF-κB activity (Fig. 2c), and it was confirmed by less translocation of p65 into nucleus after IL-1β stimulation in AMAP1-overexpressed cells (Fig. 2d). Additionally, suppression of AMAP1 by shRNA enhanced NF-κB activity (Fig. 2e) and augmented p65 translocation into the nucleus after IL-1β stimulation (Fig. 2f). Thus, AMAP1 interferes the binding of IKKβ and NEMO, and negatively regulates NF-κB.

NF-κB activation induces AMAP1 translocation to cytoplasm and augments the AMAP1-IKKβ interaction. In the experiments mentioned above, we sometimes observed that more amount of AMAP1 was detected after treatment with IL-1β (Supplementary Fig. S3a online). This observation coincides with previous report showing LPS significantly boosted AMAP1 protein levels within 15 min[23]. However, considering that 15 min is too short for synthesis or degradation of proteins, it was highly possible that AMAP1 might not be completely dissolved in cell lysis buffer because AMAP1 can be localized in lipid rafts[11–14] and nucleus[27], both of which might not be fully dissolved by 1% Triton X-100 of cell lysis buffer used in this study. Indeed, we also observed similar change of AMAP1 by LPS stimulation, but it disappeared when cells were lysed in 1% SDS cell lysis buffer (Supplementary Fig. S3b online). Therefore, we hypothesized that NF-κB activation induces AMAP1 translocation to the cytoplasm from cell membrane or nucleus, which could result in detection of more AMAP1 in cell lysate by 1% Triton X-100. As shown in Fig. 3a, fractionation by sucrose gradient indicated that IL-1β stimulation induced the translocation of AMAP1 from lipid rafts to the cytoplasm. We also performed confocal immunocytochemistry with using human umbilical vein endothelial cells (HUVECs) since HEK293T cells were not appropriate for observation of intracellular localization due to their large nuclei and thin cytoplasm. It was hard to see AMAP1 staining on cellular membrane, but we observed that IL-1β stimulation induced translocation of AMAP1 in nuclei to the cytoplasm (Fig. 3b). Finally, we repeated the sucrose gradient fractionation with HUVECs, and observed the translocation of AMAP1 from the membrane in response to IL-1β stimulation (Fig. 3c), similarly with HEK293T cells.

Considering that IKKβ is nearly entirely cytoplasmic protein[28,29], the translocation of AMAP1 to cytoplasm in response to IL-1β stimulation raised the question as to whether the activation of NF-κB would strengthen the interaction between AMAP1 and IKKβ. As shown in Fig. 3d and e, more IKKβ was co-precipitated with anti-AMAP1 antibody after IL-1β stimulation, and conversely, more AMAP1 was co-precipitated with anti-IKKβ antibody after IL-1β stimulation, revealing that NF-κB activation enhances the interaction between AMAP1 and IKKβ.

Discussion

Most biological processes require positive and negative regulatory mechanisms to maintain equilibrium. The results from this study suggest a novel mechanism for NF-κB termination and contribute new knowledge to the mechanisms of NF-κB regulation. So far, the re-synthesis of IκB proteins induced by the activation of NF-κB is a widely accepted mechanism for terminating the NF-κB response. These proteins enter the nucleus, remove NF-κB from the DNA and re-localize it to the cytosol[30–33]. Here, we show that NF-κB activation induces AMAP1 translocation to cytoplasm from nucleus or lipid rafts and accelerates binding of IKKβ and AMAP1, which inhibits the association of IKKβ and NEMO, eventually leading to inactivation of NF-κB. Taken together, AMAP1 works as an inhibitory feedback mechanism for regulating the NF-κB pathway (Fig. 4).

Recent studies have established strong support for the critical role of NF-κB in cancer. Activation of NF-κB controls multiple cellular processes in cancer, including inflammation, transformation, proliferation, angiogenesis, invasion, metastasis, chemoresistance and

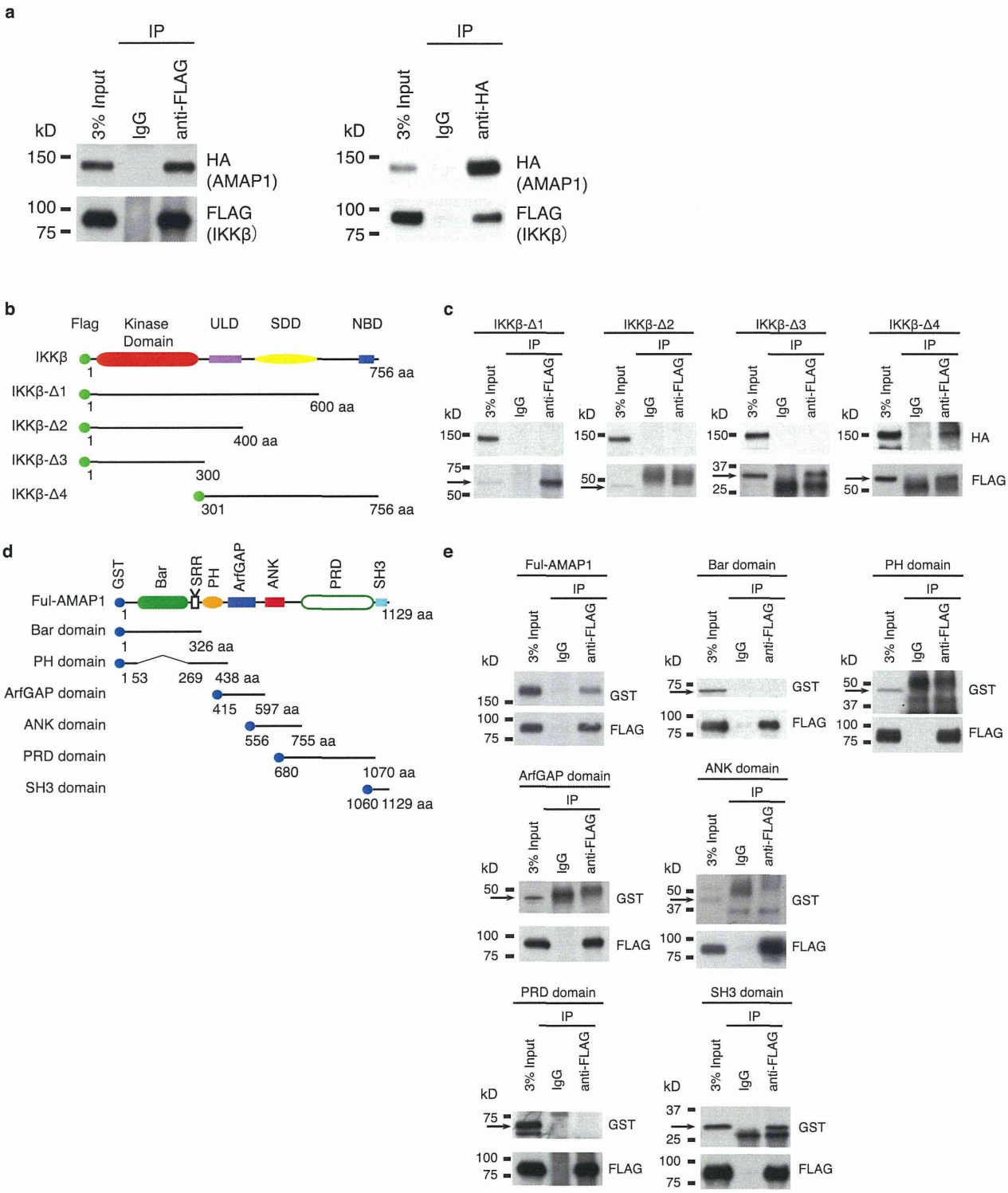

Figure 1 | AMAP1 interacts with IKKβ *in vivo*. (a) The binding of AMAP1 and IKKβ. FLAG-tagged IKKβ and HA-tagged AMAP1 were expressed in HEK293T cells and immunoprecipitated with anti-FLAG and anti-HA antibodies. AMAP1 and IKKβ were detected in the precipitate by immunoblotting with anti-FLAG and anti-HA antibodies. (b) Schematic representation of IKKβ and its mutants. (c) FLAG-tagged IKKβ/mutants and HA-tagged AMAP1 were expressed in HEK293T cells and immunoprecipitated with anti-FLAG antibody. AMAP1 was detected in the precipitate by immunoblotting with anti-HA antibody. (d) Schematic representation of AMAP1 and its mutants. (e) FLAG-tagged IKKβ and GST-tagged AMAP1/mutants were expressed in HEK293T cells and immunoprecipitated with anti-FLAG antibody. AMAP1 and its mutants were detected in the precipitate by immunoblotting with anti-GST antibody. The data shown are from one representative experiment of the three that were performed. Full-length blots are presented in Supplementary Figure 4.

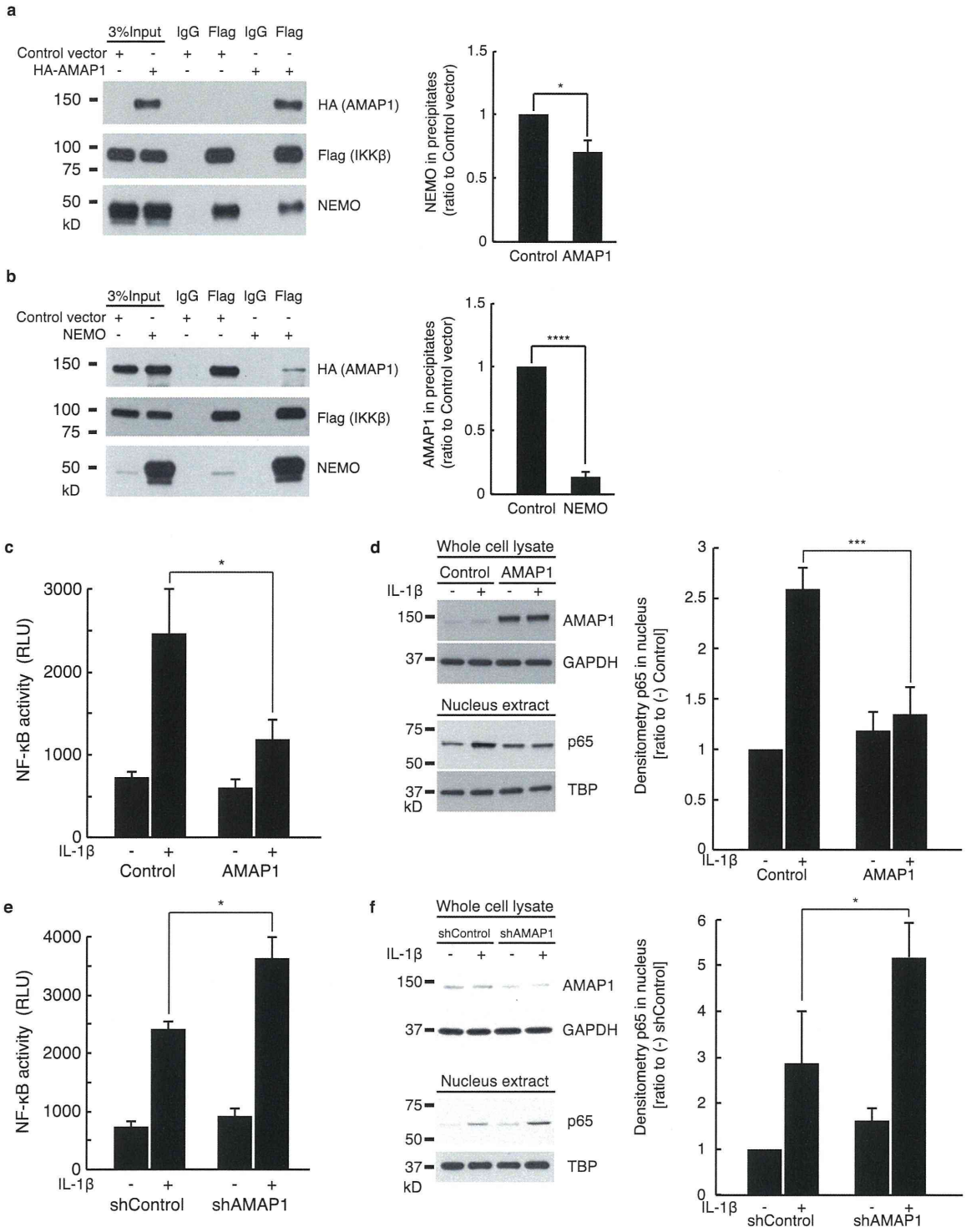

Figure 2 | AMAP1 negatively regulates NF-κB. (a) AMAP1 interfered with the association of IKKβ and NEMO. FLAG-tagged IKKβ and NEMO were expressed in HEK293T cells along with control vector or HA-tagged AMAP1 and immunoprecipitated with anti-FLAG antibody. AMAP1, IKKβ and NEMO were detected in the precipitates by immunoblotting with anti-FLAG, anti-HA and anti-NEMO antibodies. (b) The induction of NEMO reduced the interaction of IKKβ and AMAP1. FLAG-tagged IKKβ and HA-tagged AMAP1 were expressed in HEK293T cells along with control or NEMO vector and immunoprecipitated with anti-FLAG antibody. AMAP1, IKKβ and NEMO were detected in the precipitates by immunoblotting with anti-FLAG, anti-HA and anti-NEMO antibodies. (c) NF-κB activity from nuclear extracts of overexpressed AMAP1 and control cells with or without IL-1β (2.5 ng/mL) treatment for 30 min. (d) Immunoblots of AMAP1 (whole cell) and p65 (nuclear extract) of HEK293T cells transfected with pcDNA3.1-HA-AMAP1 (AMAP1 overexpression) or pcDNA3.1 (control) and the cumulative, quantitative densitometry data. (e) NF-κB activity from nuclear extracts of shAMAP1 and shControl cells with or without IL-1β (2.5 ng/mL) treatment for 30 min. (f) Immunoblots of AMAP1 (whole cell) and p65 (nuclear extract) of HEK293T cells transfected with AMAP1 shRNA (shAMAP1) or Control shRNA (shControl) with or without IL-1β (2.5 ng/mL) and the cumulative, quantitative densitometry data. The data from three independent experiments are shown. Error bar: ±SD. * $P < 0.05$, ** $P < 0.01$ in a two-sided, Student's *t*-test. Full-length blots are presented in Supplementary Figure 4.