

## Research Article

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**Abbreviations:**

CRP, c-reactive protein; HGS, handgrip strength; OA, osteoarthritis; OKS, Oxford knee score; ROM, range of motion; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index

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# Probiotics' supplementation alleviates disease severity and improves postural balance by repairing intestinal leak in patients suffering from osteoarthritis: a double-blinded clinical trial

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**Abstract**

Increased intestinal leakiness and associated systemic inflammation are potential contributors to osteoarthritis (OA) and postural imbalance in the geriatric population. To date, no successful treatment to correct postural imbalance in OA is known. We aimed to explore the effects of a multistrain probiotic upon postural imbalance in OA-affected patients. In this randomised, double-blind trial with a placebo group, 147 patients suffering from knee OA (age span = 64–75 years) were divided into placebo ( $n$  75) and probiotics ( $n$  72) study groups. Vivomix 112 billion, multistrain probiotic was given once a day for 12 weeks. The outcomes of study variables were determined first at baseline and later after 12 weeks of intervention. These were Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), knee flexion range of motion (ROM), pain intensity by visual analogue scale, handgrip strength (HGS), gait speed and balance control assessed in standing, semi-tandem and tandem stances. We determined plasma zonulin to determine intestinal leak along with c-reactive protein and 8-isoprostanes levels. A total of 136 OA patients taking placebo ( $n$  71) and probiotics ( $n$  65) were analysed. The probiotics group exhibited a reduction in pain intensity, disease severity and WOMAC scores along with improvement in balance scores, HGS and walking speed ( $P < 0.05$  for all), no change in ROM, resting pain and 8-isoprostanes levels. The correlation analysis revealed a robust association of balance scores with plasma markers of intestinal leakiness and inflammation in probiotics but not in the placebo group. Probiotics reduce postural imbalance in OA patients partly due to a reduction in intestinal leakiness.

Osteoarthritis (OA), in particular knee OA, is a common cause of disability, especially frequent among older adults. OA is described as a degenerative disease of the whole organ, showing changes in nearly all the joint apparatus, namely joint ligaments, nerves, synovial membrane, capsule, meniscus and muscles of the diseased joint<sup>(1–3)</sup>. There is mounting evidence in the literature suggesting the progression of degeneration of knee joint proprioception during the development of OA<sup>(4–6)</sup>. Therefore, joint proprioceptors (specialised nerve endings that sense position and movement) are impaired and convey inappropriate proprioceptive signals about joint movement and position<sup>(5)</sup>. Thus, defective proprioception causes loss in balance control, leading to postural imbalance, thereby augmenting the risk of falls<sup>(7–9)</sup>. Impaired proprioception is not the sole cause of postural imbalance. Several other factors, such as reduced muscle strength, knee instability, severe OA associated with pain and improper joint support<sup>(10,11)</sup>, are additional contributing factors. These patients develop adaptations during walking to compensate for poor balance control. For example, they have reduced gait speed and ROM<sup>(12)</sup>. However, the pathophysiology of postural imbalance in OA has not been explored fully and remains elusive.

Under normal, homeostatically balanced conditions, the gut microbiome maintains health and contributes to host defence. Recently, the role of altered gut microbiome/dysbiosis and associated altered immune responses along with chronic systemic inflammation was recognised as a contributing component in the pathophysiology of OA<sup>(13,14)</sup>, which has increased interest in the gut–joint axis for maintaining joint health. An intact intestinal mucosa limits the access of dangerous bacteria and their toxins into the general circulation<sup>(15)</sup>. Intestinal mucosal leakiness

and gut dysbiosis lead to widespread inflammation in reaction to the harmful bacterial endotoxins<sup>(16)</sup>. Increased intestinal leakiness can be assessed by determining plasma zonulin levels as a biomarker for mucosal barrier damage<sup>(17)</sup>. Moreover, zonulin is considered a critical controller of intestinal permeability function and is potentially involved in the pathophysiology of various chronic inflammatory diseases<sup>(18)</sup>. Furthermore, zonulin inhibitors have been proposed for treating rheumatoid arthritis, thereby reinforcing the causative role of zonulin in altered intestinal leakiness<sup>(19)</sup>. We reported elevated plasma zonulin levels associated with various diseases of old age<sup>(20–22)</sup>. Moreover, evidence suggests the association of gut microbial dysbiosis, a common reason for higher intestinal leakiness, with the severity of postural imbalance and walking problems in patients suffering from Parkinson's disease (PD)<sup>(23)</sup>. Taken together, these findings suggest the possible role of higher intestinal leakiness leading to postural imbalance. However, the existence of such a relationship in OA remains unidentified.

The use of probiotics for the correction of gut dysbiosis has emerged as a promising approach in treating several chronic metabolic diseases<sup>(24)</sup>. Moreover, therapeutic lowering of plasma zonulin with probiotics supplementation may help restore intestinal leakiness, thereby preventing the entry of harmful bacteria into circulation. Manipulation of the gut microbiota has been proposed to decrease inflammatory levels in OA related to obesity phenotype<sup>(25)</sup>. Moreover, probiotics have been suggested to modify molecular pathways related to the gut–bone axis, exerting beneficial effects on bone health and OA-like degenerative changes in joints<sup>(26,27)</sup>. However, no such role of probiotics in reducing postural imbalance related to OA has been explored. Our previous findings showed probiotics supplementation helped restore intestinal mucosal barrier function in patients suffering from chronic obstructive pulmonary disease<sup>(28)</sup>. Therefore, considering the possible link of intestinal leakiness with the development of postural imbalance in OA patients, we designed an investigation to determine the correlation of plasma zonulin with postural instability in OA patients treated with probiotics. Furthermore, we aimed to investigate the underpinning mechanism by evaluating the status of generalised inflammation and oxidative stress status in knee OA-affected patients.

## Materials and methods

### Study layout and clinical evaluation of disease severity by Oxford knee score

In this 12-week-long clinical trial, we used the double-blinded, randomised, placebo-control technique. We recruited clinically diagnosed OA patients visiting the outpatients' Department of Trauma and Orthopedic Surgery of Rehman Medical Institute (RMI), Pakistan. This study was conducted according to the guidelines laid down in the Declaration of Helsinki<sup>(29)</sup>, and all procedures involving human patients were approved by the Medical Research Ethics Committee of RMI (ref. no. RMI-HEC-13-11-75, dated: 05/01/2021). Written informed consent was obtained from all patients. The primary demographic details of patients were recorded by filling in the datasheet. The patients were evaluated by taking a thorough history and detailed physical examination. The disease diagnosis and stage were confirmed by two orthopaedic surgeons using Oxford knee score (OKS) system. It is considered a well-established standardised measurement tool to determine pain intensity and functional ability for performing

daily life activities self-reported by OA patients. OKS consists of twelve questions, graded from 0 (poorest function) to 4 (maximal function), explained in detail already<sup>(30)</sup>.

### Patient selection criteria and randomisation

We enrolled 264 men and women patients aged 64–75 years. We included symptomatic patients with unilateral or bilateral knee OA with more than 6 months of pain. We excluded patients who met one or more of the following criteria: OKS score  $\leq 6$ , intra-articular trauma or fracture, joint-related or knee replacement surgeries, intra-articular steroid injections and treatment with OA disease-modifying agents. Moreover, patients with gastrointestinal-related diseases taking probiotics or antibiotics were excluded based on their past medical and drug-related history. All women were postmenopausal and not taking any hormonal or bone-modifying medications. All patients had the same ethnic and geographic background.

A sample size calculation was performed to determine the optimal group size for this randomised control trial. To detect the anticipated 5% change in plasma zonulin levels with a desired statistical power of 80% ( $\beta = 0.2$ ) and a significance level of 0.05 ( $\alpha$ ), sixty-three participants per group were determined to be necessary. We assessed 264 enrolled patients; 147 fulfilled the inclusion criteria. One hundred and seventeen patients were excluded since fifty-nine did not meet the criteria, fifty-three did not consent to participate and five were excluded for variable reasons. Therefore, an independent analyst, not engaged in the disease assessment/diagnosis and determination of study outcome variables, performed computer randomisation of 147 recruited OA patients. Likewise, the researchers were unaware of the randomisation process. Patients were allocated randomised codes to receive the intervention. Therefore, allocation to placebo ( $n = 75$ ) and probiotics ( $n = 72$ ) groups was completely blinded. The intervention continued for 3 months (12 weeks), and during the follow-up period, none of the patients were lost to follow-up in the placebo group, and only one patient was lost in the probiotics group. Moreover, four and six patients discontinued intervention from the placebo and probiotics groups, respectively, and were excluded from the trial. Upon the completion of the trial, 136 patients in the placebo ( $n = 71$ ) and probiotics ( $n = 65$ ) groups were analysed for outcome measurements (Fig. 1).

### Probiotic and placebo products

In our previous study, we have already explained the treatment product details<sup>(31)</sup>. Patients received either probiotic or placebo capsules once daily for 12 weeks. Vivomix 112 billion is a high-potency probiotic capsule containing 112 billion live bacteria from eight specific strains. These strains included *Streptococcus thermophilus* DSM 24731, *Bifidobacterium longum* DSM 24736, *Bifidobacterium breve* DSM 24732 and DSM 24737, *Lactobacillus* strains DSM 24735, DSM 24730, DSM 24733 and *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 24734. The capsule also contains maltose as an inactive ingredient and silicon dioxide as an anti-caking agent (Vivomix). Placebo capsules were isoenergetic-matched ones containing no biologically active ingredient. Both placebo and probiotics were white powders that dissolved in water and were provided to patients in similar opaque containers with appropriate storage instructions. The study was designed to investigate the effect of probiotics independent of any lifestyle modifications such as diet or physical activity; therefore, patients were advised to maintain their routine lifestyle. Moreover, patients

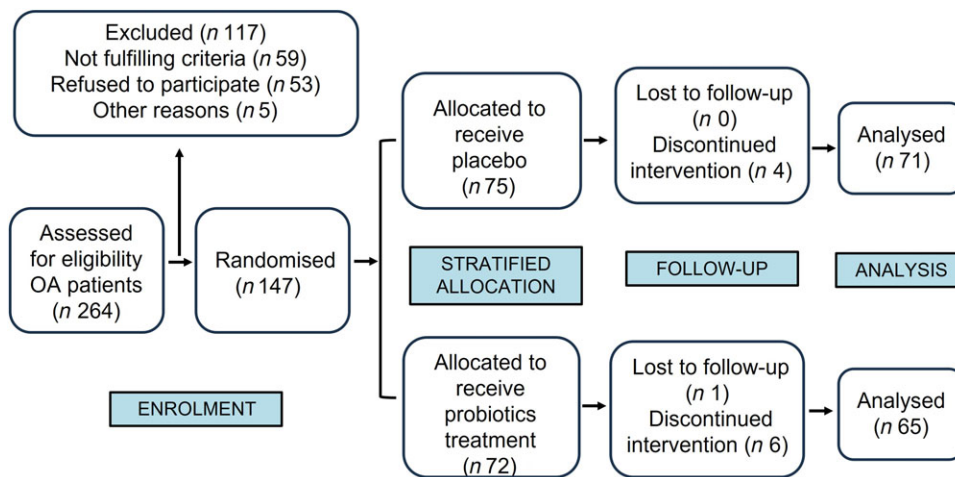


Figure 1. Flow chart of the study.

were instructed to continue the management of OA, which was already ongoing at the beginning of the trial.

#### Assessment of knee joint range of motion

Firstly, at the baseline and later after 12 weeks of treatment, knee flexion measurements were performed to determine the knee joint ROM. Measurements were performed with patients lying on the examination couch comfortably, following the protocol described<sup>(32)</sup>. A digital electronic goniometer (Meloq) was used by trained staff to measure the ROM of the OA-affected joint without any forceful movements. We recorded three consecutive readings with brief periods of relaxation, and a mean value was considered for data analysis.

#### Assessment of knee pain intensity by visual analogue scale

Patients rated their pain intensity using visual analogue scale (VAS), ranging from no pain (0) to worst possible pain (10) for knee pain rating. We followed the pain measurement scale already described elsewhere<sup>(33)</sup>. Pain intensity measurements were performed at the start (baseline) and end (after 12 weeks) of the trial, and patients were instructed to discontinue any analgesics 3 d before VAS measurements to avoid any masking effects of drugs. At each visit, patients were instructed to finish two VAS measurements, one at rest and the second while walking, to reflect pain intensity in resting and active states of the diseased joint.

#### Assessment of pain status and function by Western Ontario and McMaster Universities Osteoarthritis Index

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is a broadly utilised OA-specific questionnaire based on the self-reporting of symptoms by patients to evaluate underlying disease activity. The questionnaire has three sections, and the WOMAC score spans between 0 and 96 points. The three sections comprise questions for pain (total points = 20), stiffness (total points = 8) and functional deficiency (total points = 68). The total WOMAC count is obtained by adding the points from three categories; higher index values suggest severe symptoms and diminished joint function and vice versa<sup>(34)</sup>. In this trial, we measured the WOMAC index scores of our study patients at baseline and after 12 weeks of intervention.

#### Handgrip strength and gait speed measurements

Handgrip strength (HGS), an important tool to measure muscle strength, is associated with knee OA, especially with a classic radiographic feature of the disease, that is, joint space narrowing<sup>(35)</sup>. We measured the HGS of our study patients by using a numerical hand dynamometer (CAMRY), following the protocol already described by us<sup>(20,28)</sup>. In brief, patients had to sit comfortably in the chair and hold the dynamometer in the dominant hand, squeezing it with maximum strength with the elbow flexed at 90°. The movements were ensured to be non-jerky, and three consecutive readings were recorded with brief intervals of relaxation in between. A mean value was calculated and used for analysis. Next, we determined the functional capacity of our study patients by recording their gait speed, following the protocol already described by us<sup>(36)</sup>.

#### Assessment of postural balance

At baseline and 12 weeks after the intervention, we determined the postural stability of our patients by assessing their body balance in standing posture. Patients were made to stand upright without support, and we assessed three balancing positions. Patients were made to put their feet together to assess side-by-side balance, a semi-tandem and tandem stance for 10 s. The scoring system had a range of 0 (poor performance) to 4 (maximum performance), as described already<sup>(37)</sup>. One point was given to the patients able to balance their body with feet together and in semi-tandem positions for 10 s. Patients unable to maintain or not willing to try to maintain postural balance were allocated no points. Two points were allocated to patients capable of maintaining a fully tandem position for 10 s and able to maintain for 3–9.99 s were given 1 point; however, if the time for maintaining this position was less than 3 s, then no points were allocated.

#### Circulating biomarkers' assessment

Three millilitres of blood were drawn from the patients and stored in suitable vacutainers to obtain platelet-free plasma. Plasma was stored in Eppendorf tubes at  $-80^{\circ}\text{C}$ . We used commercially available ELISA kits and followed the manufacturer's protocol to determine levels of plasma zonulin (Cat # K5601, Immundiagnostik AG), c-reactive proteins (CRP) (R&D Systems) and 8-isoprostanes (Cayman Chemical), as described previously<sup>(21)</sup>.



### Statistical evaluation

The anthropometric measurements were given as mean and standard deviation since the data met the criterion of normal distribution. Group-wise comparisons were done by using paired *t* tests, and regression analysis was performed to study the correlation between various study variables. We used GraphPad Prism 8 (Graph Stats Technologies) for data analysis and creating graphs. A *P*-value of < 0.05 was regarded as statistically significant. \**P* < 0.05 *v.* baseline value of the same group.

## Results

### Basic demographic and medical features of patients

We analysed 136 patients in the placebo (*n* 71) and probiotics (*n* 65) groups for the outcome measures. The study patients were age, BMI and sex-matched and had similar ethnicity (Table 1). OA patients in both placebo and probiotics groups had comparable disease duration and percentages of affected knee joints. All the OA patients in this trial had almost the same WOMAC index scores, severity of the disease according to OKS, pain intensity according to VAS and degree of knee flexion according to ROM. At the baseline, no significant difference existed between the clinical and biochemical parameters of the study patients. After 12 weeks of intervention, no changes were found in the placebo group in the studied outcome variables. Conversely, OA patients following treatment for 12 weeks with probiotics exhibited a marked reduction in OA-related symptoms and overall disease severity (*P* < 0.05). There was a substantial reduction in WOMAC index scores, pain intensity while walking and consumption of analgesics (non-steroidal anti-inflammatory drugs) following treatment with probiotics (*P* < 0.05 for all). OA-affected patients also showed improvement in physical performance as displayed by improvement in balance scores, HGS and walking speed (*P* < 0.05 for all). Moreover, plasma zonulin and CRP levels were found to be reduced in this group (all *P* < 0.05). However, we did not find any improvement in pain intensity at rest according to VAS, knee flexion ROM of diseased joints and plasma 8-isoprostanes levels among patients in the probiotics group.

### Changes in Oxford knee score, physical capacity and circulating biomarkers according to balance scores in osteoarthritis patients treated with probiotics

Next, we categorised the study patients based on their balance scores into three groups with balance scores of 4, 3 and ≤ 2 to investigate whether study outcomes were affected by the altered postural balance of OA patients taking either placebo or probiotics. OA patients within three balance score groups in both placebo and probiotics groups showed similar OKS scores at baseline. After 12 weeks of trial, no change was observed in the OKS scores of patients in the placebo group. Conversely, the OA-affected patients having 4, 3 and ≤ 2 balance scores, after taking probiotics, showed a significant improvement in their OKS scores reflected as a reduction in disease severity when compared with baseline (*P* < 0.05 for all) (Fig. 2(a)). Additionally, the OA patients with balance scores 3 and ≤ 2 exhibited an increase in gait speed following treatment with probiotics in comparison with baseline (both *P* < 0.05) (Fig. 2(b)). Similarly, OA patients with a balance score of 3 after probiotics supplementation showed an improvement in HGS as compared with the baseline (*P* < 0.05) (Fig. 2(c)). Therefore, OA patients with a baseline balance score of 3 showed

an overall improvement in functional capacity and a reduction in disease severity after probiotics treatment. The patients in the placebo group showed no changes in disease severity, gait speed and HGS after 12 weeks compared with baseline.

We next investigated whether supplementation with probiotics affected intestinal leakiness and its associated inflammation in OA patients with various categories of balance scores. After 12 weeks of probiotics treatment, the OA patients within all three categories of balance scores showed a significant reduction in plasma zonulin (all *P* < 0.05) (Fig. 2(d)) and CRP levels (all *P* < 0.05) (Fig. 2(e)), in comparison with the baseline, with no such changes observed in the placebo group. Moreover, plasma 8-isoprostanes levels remained unchanged in both placebo and probiotics groups (Fig. 2(f)).

### Changes in balance scores, physical capacity and circulating biomarkers according to Oxford knee scores in osteoarthritis patients treated with probiotics

Next, we questioned whether disease severity affected the study outcomes in OA patients taking either placebo or probiotics for 12 weeks. Therefore, we grouped the patients into three categories (mild, moderate and severe) by using the OKS scoring system. Patients with mild, moderate and severe OA showed comparable balance scores at baseline in the study's placebo and probiotics groups. Following the intervention of 12 weeks, patients in the placebo group showed no changes in the balance scores compared with baseline. On the contrary, OA patients in moderate and severe categories taking probiotics treatment exhibited an improvement in their balance scores as compared with baseline (both *P* < 0.05) (Fig. 3(a)). Moreover, the gait speed of patients with mild and moderate OA improved as compared with baseline following probiotics treatment (*P* < 0.05) (Fig. 3(b)). HGS was improved in patients suffering from severe OA after 12 weeks of probiotics supplementation as compared with baseline (*P* < 0.05) (Fig. 3(c)). However, no changes in postural balance and functional capacity were observed in the OA patients taking a placebo. We further investigated whether probiotics affected plasma biomarkers among OA patients with varying degrees of disease severity. OA patients with the mild, moderate and severe disease showed a reduction in plasma zonulin (*P* < 0.05) (Fig. 3(d)), CRP levels were reduced among patients affected by OA with moderate and severe degrees (both *P* < 0.05) (Fig. 3(e)) and no change observed in plasma 8-isoprostanes levels (Fig. 2(f)) when compared with baseline values. However, participants in the placebo group showed no changes in plasma biomarkers levels.

### Correlation of plasma biomarkers with disease severity, physical ability and postural balance in osteoarthritis-affected patients treated with probiotics

Lastly, we determined the correlations of plasma zonulin, an intestinal permeability marker, CRP an inflammatory marker and 8-isoprostanes, a marker of systemic oxidative stress with OA-related disease severity, physical capacity and postural imbalance in placebo and probiotics groups (Table 2). We found a significant correlation between WOMAC scores, gait speed, OKS and balance scores with plasma zonulin levels in OA patients after probiotics treatment for 12 weeks. A robust correlation was observed with balance ( $r^2 = 0.435$ ) and WOMAC index scores ( $r^2 = 0.309$ ). We observed a significant correlation of plasma CRP levels with WOMAC scores, HGS, OKS and balance scores among OA patients taking probiotics. However, a robust association was

**Table 1.** Demographic characteristics of the OA patients corresponding to the placebo and probiotics treatment over a period of 12 weeks (Numbers and percentages; mean values and standard deviations)

	Placebo				Probiotics				
	Baseline		12 weeks		Baseline		12 weeks		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age (years)	69.1	4.6	69.2	3.9	69.3	5.5	69.5	5.1	
BMI (kg/m <sup>2</sup> )	25.38	5.5	26.68	4.8	25.21	4.2	24.96	3.5	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Sex <i>n</i> (%)									
Male	24	32	21	29.5	23	31.9	19	29	
Female	51	68	50	70.4	49	68	45	69.2	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Duration of arthritis (years)	12.5	4.5	12.7	4.2	13.5	7.2	13	7.5	
Affected joint side (%)		%		%		%		%	
Left	47.7		47.7		47.3		47.3		
Right	52.3		52.3		52.1		52.1		
Pain intensity (VAS)		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Resting		4.25	3.6	4.28	3.2	4.58	3.3	4.21	2.5
Walking		6.8	4.6	6.9	3.5	7.6	2.8	6.1	2.2*
		%		%		%		%	
WOMAC index		47.2		47.5		47.8		44.5*	
Taking NSAID for OA treatment (%)		74.5		74.5		75.6		72.8*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
OKS	23.80	5.46	23.20	5.21	24.68	6.85	28.80	7.55*	
Balance scores	2.39	0.34	2.25	0.50	2.36	0.52	2.55	0.40*	
Knee flexion ROM	121.5	6.8	121.3	6.5	122.2	5.7	123.7	5.2	
HGS (Kgs)	17.22	2.65	17.14	2.81	18.21	3.25	19.56	3.56*	
Gait speed (m/s)	0.94	0.10	0.92	0.15	0.95	0.20	1.05	0.21*	
Plasma zonulin (ng/ml)	2.68	0.45	2.56	0.36	2.73	0.41	2.25	0.25*	
8-isoprostanes (pg/ml)	73.20	30.2	82.50	28.80	75.40	31.2	85.24	30.5	
CRP (mg/l)	2.67	0.45	2.85	0.52	2.75	0.60	2.30	0.50*	

OA, osteoarthritis; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Arthritis Index; NSAID, non-steroidal anti-inflammatory drugs; OKS, Oxford knee score; ROM, range of motion; HGS, handgrip strength; CRP, c-reactive protein.

Values are expressed as mean  $\pm$  sd, paired *t* test.

\**P* < 0.05 v. baseline of the same group (*n* 65–71/group).

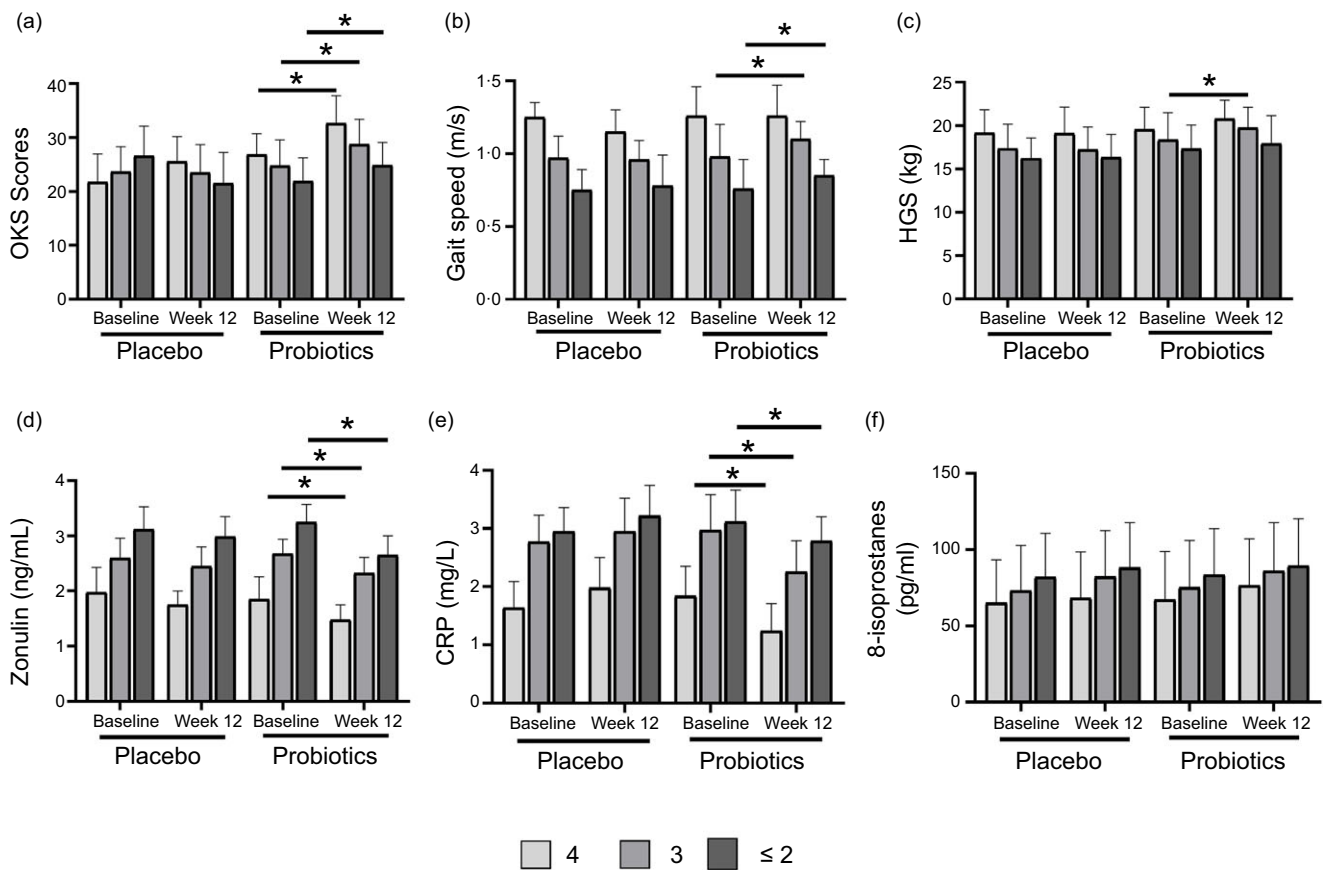
found with balance scores ( $r^2 = 0.412$ ). Plasma 8-isoprostanes levels were significantly correlated with WOMAC scores, HGS and OKS scores in OA patients taking probiotics, with the highest association observed with HGS ( $r^2 = 0.375$ ).

## Discussion

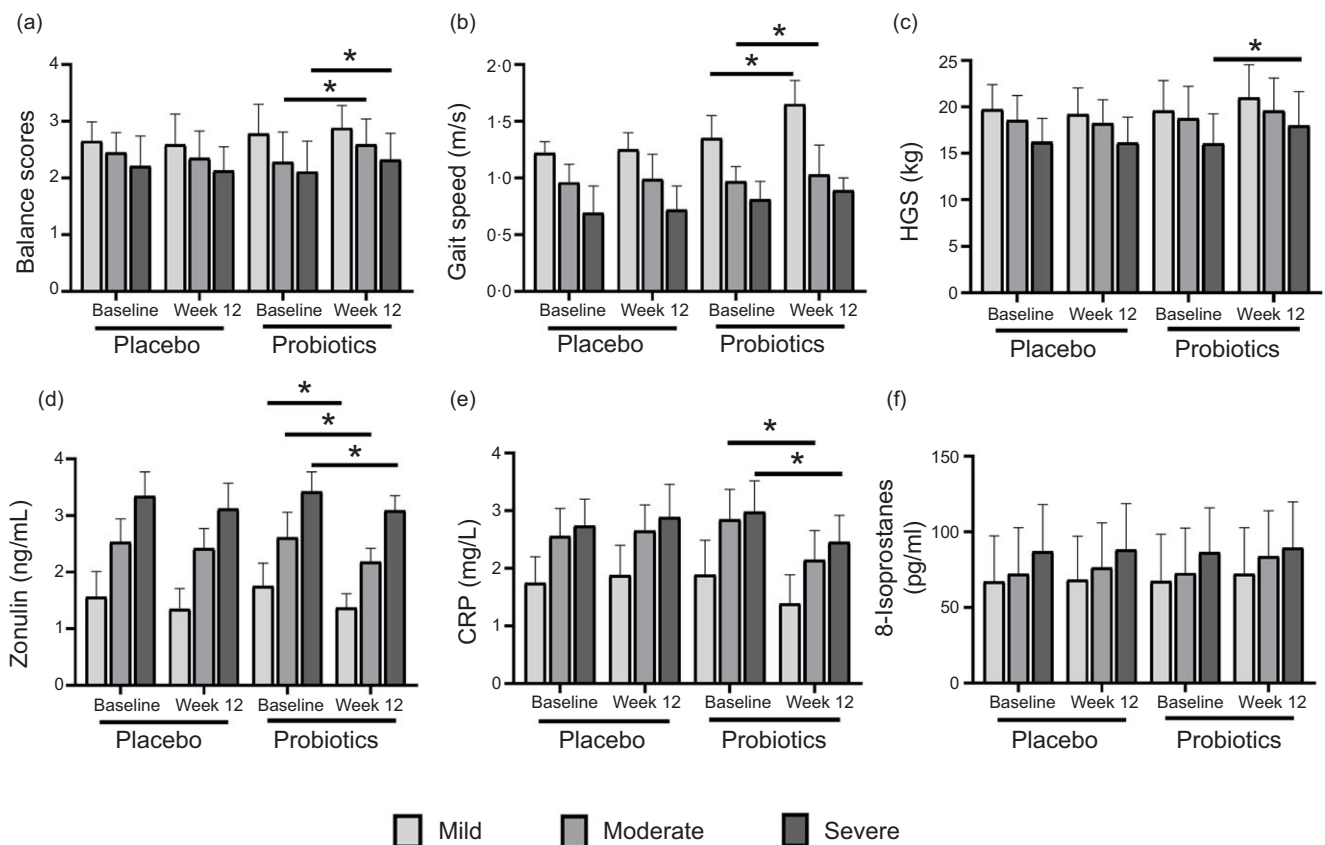
Our results indicate that the administration of probiotics reduced pain and disease severity in patients suffering from knee OA. These effects were accompanied by improvement in gait speed and HGS in the probiotics group after 12 weeks of intervention as compared with the baseline. There was a significant reduction in plasma zonulin and CRP levels, while no changes were observed in 8-isoprostanes levels following probiotics supplementation in OA patients. Moreover, we report an improvement in balance scores in

these patients. A reduction in plasma zonulin and inflammatory markers may partly describe intestinal mucosal repair as the possible underlying mechanism for the beneficial effects of probiotics in OA patients with postural imbalance. Additionally, plasma zonulin can potentially serve as a marker to assess postural imbalance and functional compromise in these patients.

Our findings support the existence and functioning of the gut–joint axis for the maintenance of joint-related health<sup>(38)</sup>. However, the correlation of intestinal permeability and associated gut dysbiosis with postural imbalance in OA was deficient. We have reported that probiotics intake can alleviate postural imbalance in older adults with OA. The role of gut dysbiosis in developing postural instability and gait difficulty phenotypes has been reported in neurodegenerative diseases, such as PD<sup>(39)</sup> and multiple sclerosis<sup>(40)</sup>. Our observations extend the role of gut



**Figure 2.** (a) OKS scores, (b) gait speed, (c) HGS, (d) plasma zonulin, (e) CRP and (f) 8-isoprostanes levels in placebo (*n* 71) and probiotics groups (*n* 65) according to balance scores, \**P* < 0.05. OKS, Oxford knee score; HGS, handgrip strength; CRP, c-reactive protein; OA, osteoarthritis.



**Figure 3.** (a) OKS scores, (b) gait speed, (c) HGS, (d) plasma zonulin, (e) CRP and (f) 8-isoprostanes levels in placebo (*n* 71) and probiotics groups (*n* 65) according to balance scores, \**P* < 0.05. OKS, Oxford knee score; HGS, handgrip strength; CRP, c-reactive protein; OA, osteoarthritis.

**Table 2.** Coefficients of determination of plasma zonulin, CRP and 8-isoprostanes with indexes of WOMAC, functional performance, disease severity and balance scores in OA patients according to placebo and probiotic treatments

	Placebo		Probiotics	
	Baseline	12 weeks	Baseline	12 weeks
<b>Zonulin</b>				
WOMAC	0.156	0.176	0.256*	0.309*
HGS	0.276*	0.298*	0.319*	0.415*
Gait speed	0.064	0.068	0.104	0.128*
OKS	0.106	0.101	0.146*	0.241*
Balance scores	0.265*	0.287*	0.351*	0.435*
<b>CRP</b>				
WOMAC	0.245*	0.306*	0.389*	0.399*
HGS	0.276*	0.285*	0.329*	0.388*
Gait speed	0.078	0.085	0.134*	0.108
OKS	0.112	0.097	0.159	0.257*
Balance scores	0.254*	0.187*	0.399*	0.412*
<b>8-isoprostanes</b>				
WOMAC	0.124	0.098	0.126	0.256*
HGS	0.265*	0.248*	0.308*	0.375*
Gait speed	0.112	0.098	0.115*	0.126
OKS	0.075	0.099	0.153*	0.177*
Balance scores	0.115	0.179*	0.059	0.086

CRP, c-reactive protein; WOMAC, Western Ontario and McMaster Universities Arthritis Index; OA, osteoarthritis; HGS, handgrip strength; OKS, Oxford knee score.  
\* $P < 0.05$  ( $n = 65-71$ /group).

dysbiosis in developing postural imbalance in OA patients. Moreover, beneficial effects of probiotics in improving postural instability and gait difficulty have been observed in a mouse model of PD, suggesting the role of the gut-brain axis in preventing deterioration of motor functions in these patients<sup>(41)</sup>, which further supports our findings.

The postural imbalance is coupled with declining muscle strength, proprioception and neural control of muscles and joints. Although there is very limited evidence regarding the association of increased intestinal leakiness with postural imbalance in OA patients, this mechanistic factor has been proposed in developing several other chronic inflammatory diseases. High plasma zonulin, a marker of intestinal leakiness, has been reported to be associated with a decline in muscle strength and functional performance. Furthermore, we have previously reported the association of high plasma zonulin levels with muscle weakness and impaired functional capacity in several age-related diseases<sup>(20,21,42)</sup>. Several previous studies have also reported the beneficial effects of probiotics intake in reducing plasma zonulin, discussed in detail elsewhere<sup>(43)</sup>. These previous reports align with our findings that probiotics supplementation lowers plasma zonulin levels and is partly involved in intestinal repair, thereby improving postural stability in OA patients.

Previous studies have reported the beneficial effects of probiotics use in knee OA patients and have suggested this effect to be due to suppression of inflammation. Moreover, clinical

studies have shown lower CRP levels in OA patients, a marker of inflammation following probiotics treatment<sup>(44)</sup>. These observations support the findings of our trial, where probiotics lowered CRP levels among patients in the probiotics group. We found improved HGS after probiotics intake, as observed previously<sup>(45)</sup>. We have also reported a reduction in pain sensitivity among the OA patients following probiotics use, which is in line with previously reported randomised controlled trials<sup>(46)</sup>. Interestingly, in an OA experimental model, lactobacilli's beneficial effects on cartilage health have been reported<sup>(47)</sup>, which was also the component of our multistrain probiotic. Therefore, the marked reduction in pain sensitivity and improvement in disease severity observed after probiotics intervention in our study cohort reinforces the existence of the gut-joint axis and its potential to be manipulated therapeutically.

Since relevant studies on this topic are very limited, we suggest replication on a larger sample size. Additionally, understanding the role of individual strains of bacteria in improving joint health warrants more investigations. Furthermore, detailed molecular analysis of the blood of patients taking probiotics can help identify individual bacterial components responsible for improving the balance status of these patients.

This study has certain limitations. We need to expand our understanding by conducting such studies on a varied population regarding ethnicity and background. We have not considered any changes in the lifestyle and food habits of our studied population during the trial, which can be a potential confounding factor. We have not studied microbiome composition, which can potentially modify probiotics' effects. The major strength of this trial was its randomisation to rule out the investigator's bias. Moreover, our patients had the same ethnic and geographic background, thereby reducing gut microbiome variability<sup>(48)</sup>. The study tools were simple, inexpensive, convenient to use and easily accessible in any clinical set-up.

## Conclusions

Altogether, our findings reinforce the impact of the gut-joint axis and its potential therapeutic manipulation on the pathophysiology of postural imbalance in OA patients. We have proposed that increased intestinal leakiness and associated widespread inflammation augment OA symptoms, mainly pain and functional performance. Moreover, a leaky gut is a primary contributing factor in accelerating postural instability in OA patients. This is the first clinical study to report the beneficial effects of probiotics intake in improving balance in OA patients. We propose high plasma zonulin levels as a potential biomarker for the clinical evaluation of postural balance in OA patients. Lastly, our work suggests the beneficial effects of probiotics to be used as a therapeutic option to reduce postural imbalance in old-age patients suffering from OA.

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The data are available from the corresponding author upon reasonable request.

## References

- Loeser RF, Goldring SR, Scanzello CR, *et al.* (2012) Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum* **64**, 1697–1707.
- Hunter DJ & Bierma-Zeinstra S (2019) Osteoarthritis. *Lancet* **393**, 1745–1759.
- Brandt KD, Radin EL, Dieppe PA, *et al.* (2006) Yet more evidence that osteoarthritis is not a cartilage disease. *Ann Rheum Dis* **65**, 1261–1264.
- Sharma L, Pai YC, Holtkamp K, *et al.* (1997) Is knee joint proprioception worse in the arthritic knee *v.* the unaffected knee in unilateral knee osteoarthritis? *Arthritis Rheum* **40**, 1518–1525.
- Al-Dadah O, Shepstone L & Donell ST (2020) Proprioception deficiency in articular cartilage lesions of the knee. *Knee Surg Relat Res* **32**, 25.
- Knoop J, Steultjens MP, van der Leeden M, *et al.* (2011) Proprioception in knee osteoarthritis: a narrative review. *Osteoarthritis Cartilage* **19**, 381–388.
- Dore AL, Golightly YM, Mercer VS, *et al.* (2015) Lower-extremity osteoarthritis and the risk of falls in a community-based longitudinal study of adults with and without osteoarthritis. *Arthritis Care Res (Hoboken)* **67**, 633–639.
- Anderson ML, Allen KD, Golightly YM, *et al.* (2019) Fall risk and utilization of balance training for adults with symptomatic knee osteoarthritis: secondary analysis from a randomized clinical trial. *J Geriatr Phys Ther* **42**, E39–E44.
- Levinger P, Menz HB, Wee E, *et al.* (2011) Physiological risk factors for falls in people with knee osteoarthritis before and early after knee replacement surgery. *Knee Surg Sports Traumatol Arthrosc* **19**, 1082–1089.
- Manlapaz DG, Sole G, Jayakaran P, *et al.* (2019) Risk factors for falls in adults with knee osteoarthritis: a systematic review. *PM R* **11**, 745–757.
- Hatfield GL, Morrison A, Wenman M, *et al.* (2016) Clinical tests of standing balance in the knee osteoarthritis population: systematic review and meta-analysis. *Phys Ther* **96**, 324–337.
- Lewek MD, Rudolph KS & Snyder-Mackler L (2004) Control of frontal plane knee laxity during gait in patients with medial compartment knee osteoarthritis. *Osteoarthritis Cartilage* **12**, 745–751.
- Tan TC, Chong TKY, Low AHL, *et al.* (2021) Microbiome and osteoarthritis: new insights from animal and human studies. *Int J Rheum Dis* **24**, 984–1003.
- Binvignat M, Sokol H, Mariotti-Ferrandiz E, *et al.* (2021) Osteoarthritis and gut microbiome. *Joint Bone Spine* **88**, 105203.
- Fasano A (2020) All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. *F1000Res* **9**, F1000.
- Harte AL, da Silva NF, Creely SJ, *et al.* (2010) Elevated endotoxin levels in non-alcoholic fatty liver disease. *J Inflamm (Lond)* **7**, 15.
- Moreno-Navarrete JM, Sabater M, Ortega F, *et al.* (2012) Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. *PLoS One* **7**, e37160.
- Sturgeon C & Fasano A (2016) Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers* **4**, e1251384.
- Tajik N, Frech M, Schulz O, *et al.* (2020) Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. *Nat Commun* **11**, 1995.
- Karim A, Muhammad T, Ustrana S, *et al.* (2021) Intestinal permeability marker zonulin as a predictor of Sarcopenia in chronic obstructive pulmonary disease. *Respir Med* **189**, 106662.
- Ahmad F, Karim A, Khan J, *et al.* (2022) Plasma zonulin correlates with cardiac dysfunction and poor physical performance in patients with chronic heart failure. *Life Sci* **311**, 121150.
- Ahmad F, Karim A, Khan J, *et al.* (2023) Plasma galectin-3 and H-FABP correlate with poor physical performance in patients with congestive heart failure. *Exp Biol Med (Maywood)* **248**, 532–540.
- Scheperjans F, Aho V, Pereira PA, *et al.* (2015) Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord* **30**, 350–358.
- Brunkwall L & Orho-Melander M (2017) The gut microbiome as a target for prevention and treatment of hyperglycaemia in type 2 diabetes: from current human evidence to future possibilities. *Diabetologia* **60**, 943–951.
- Schott EM, Farnsworth CW, Grier A, *et al.* (2018) Targeting the gut microbiome to treat the osteoarthritis of obesity. *JCI Insight* **3**, e95997.
- de Sire A, de Sire R, Curci C, *et al.* (2022) Role of dietary supplements and probiotics in modulating microbiota and bone health: the gut-bone axis. *Cells* **11**, 743.
- de Sire A, Marotta N, Marinario C, *et al.* (2021) Role of physical exercise and nutraceuticals in modulating molecular pathways of osteoarthritis. *Int J Mol Sci* **22**, 5722.
- Karim A, Muhammad T, Shah I, *et al.* (2022) A multistrain probiotic reduces sarcopenia by modulating Wnt signaling biomarkers in patients with chronic heart failure. *J Cardiol* **80**, 449–455.
- World Medical A (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* **310**, 2191–2194.
- Dawson J, Fitzpatrick R, Murray D, *et al.* (1998) Questionnaire on the perceptions of patients about total knee replacement. *J Bone Joint Surg Br* **80**, 63–69.
- Karim A, Muhammad T, Shahid Iqbal M, *et al.* (2022) A multistrain probiotic improves handgrip strength and functional capacity in patients with COPD: a randomized controlled trial. *Arch Gerontol Geriatr* **102**, 104721.
- Oka T, Wada O, Asai T, *et al.* (2020) Importance of knee flexion range of motion during the acute phase after total knee arthroplasty. *Phys Ther Res* **23**, 143–148.
- Rindone JP, Hiller D, Collacott E, *et al.* (2000) Randomized, controlled trial of glucosamine for treating osteoarthritis of the knee. *West J Med* **172**, 91–94.
- Bellamy N & Buchanan WW (1986) A preliminary evaluation of the dimensionality and clinical importance of pain and disability in osteoarthritis of the hip and knee. *Clin Rheumatol* **5**, 231–241.
- Wen L, Shin MH, Kang JH, *et al.* (2017) Association between grip strength and hand and knee radiographic osteoarthritis in Korean adults: data from the Dong-gu study. *PLoS One* **12**, e0185343.
- Qaisar R, Karim A, Muhammad T, *et al.* (2020) Circulating biomarkers of accelerated sarcopenia in respiratory diseases. *Biology (Basel)* **9**, 322.
- Ramirez-Velez R, Perez-Sousa MA, Venegas-Sanabria LC, *et al.* (2020) Normative values for the Short Physical Performance Battery (SPPB) and their association with anthropometric variables in older Colombian adults. The SABE Study, 2015. *Front Med (Lausanne)* **7**, 52.
- Gleason B, Chisari E & Parvizi J (2022) Osteoarthritis can also start in the gut: the gut-joint axis. *Indian J Orthop* **56**, 1150–1155.
- Mulak A & Bonaz B (2015) Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol* **21**, 10609–10620.
- Nouri M, Bredberg A, Westrom B, *et al.* (2014) Intestinal barrier dysfunction develops at the onset of experimental autoimmune encephalomyelitis, and can be induced by adoptive transfer of auto-reactive T cells. *PLoS One* **9**, e106335.
- Hsieh TH, Kuo CW, Hsieh KH, *et al.* (2020) Probiotics alleviate the progressive deterioration of motor functions in a mouse model of Parkinson's Disease. *Brain Sci* **10**, 206.
- Karim A, Iqbal MS, Muhammad T, *et al.* (2022) Elevated plasma zonulin and CAF22 are correlated with sarcopenia and functional dependency at various stages of Alzheimer's diseases. *Neurosci Res* **184**, 47–53.
- Ramezani Ahmadi A, Sadeghian M, Alipour M, *et al.* (2020) The effects of probiotic/synbiotic on serum level of zonulin as a biomarker of intestinal permeability: a systematic review and meta-analysis. *Iran J Public Health* **49**, 1222–1231.
- Lei M, Guo C, Wang D, *et al.* (2017) The effect of probiotic Lactobacillus casei Shirota on knee osteoarthritis: a randomised double-blind, placebo-controlled clinical trial. *Benef Microbes* **8**, 697–703.



45. Prokopidis K, Giannos P, Kirwan R, *et al.* (2023) Impact of probiotics on muscle mass, muscle strength and lean mass: a systematic review and meta-analysis of randomized controlled trials. *J Cachexia Sarcopenia Muscle* **14**, 30–44.
46. Pedersini P, Savoldi M, Berjano P, *et al.* (2021) A probiotic intervention on pain hypersensitivity and microbiota composition in patients with osteoarthritis pain: study protocol for a randomized controlled trial. *Arch Rheumatol* **36**, 296–301.
47. O-Sullivan I, Natarajan Anbazhagan A, Singh G, *et al.* (2022) Lactobacillus acidophilus mitigates osteoarthritis-associated pain, cartilage disintegration and gut microbiota dysbiosis in an experimental Murine OA model. *Biomedicines* **10**, 1298.
48. Gaulke CA & Sharpton TJ (2018) The influence of ethnicity and geography on human gut microbiome composition. *Nat Med* **24**, 1495–1496.