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EDITORIAL

Arthroscopic capsular release and manipulation under anaesthesia for frozen shoulders: A hot topic

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Abstract

A frozen shoulder is a common cause of shoulder pain and stiffness. The etiology and pathology of frozen shoulders is not fully understood yet. Frozen shoulder is characterized by a decrease in intra-articular volume and capsular compliance. This can lead to significant limitations in daily life. The majority of the patients can be treated conservatively, with functional recovery to be expected in two to three years. However, if conservative treatment fails, manipulation under anaesthesia and arthroscopic capsular release can both be considered as appropriate treatments. Manipulation is a traditionally well-established technique but in recent years it seems that arthroscopic capsular release has gained popularity. Manipulation is a relative time efficient and technically low-demanding procedure in which the glenohumeral joint is forced into different directions under general anaesthesia to release the capsular contracture, thereby increasing the range of motion of the joint. In arthroscopic capsular release the glenohumeral capsule can be released in a more controlled manner under direct vision. There are no prospective comparative trials available to display superiority of one procedure over the other. In addition, the optimal timing of both these interventions still has to be determined. An overview of the literature concerning this topic and a description of both procedures with its own advantages and disadvantages is provided.

Key words: Frozen shoulder; Adhesive capsulitis; Manipulation; Arthroscopy; Capsular release; Shoulder; Shoulder stiffness

Core tip: A frozen shoulder is a common cause of shoulder pain and stiffness, which is characterized by a decrease in intra-articular volume and capsular compliance. If conservative treatment fails, manipulation under anaesthesia and arthroscopic capsular release can both be considered as appropriate treatments. An overview of the literature concerning this topic and a description of both procedures with its own advantages

and disadvantages is provided.

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EPIDEMIOLOGY

A frozen shoulder is a commonly encountered condition in the orthopaedic surgeons' practice. Pain and restricted range of motion of the shoulder may lead to disability and a decrease in quality of life. In 1872 Duplay[1] described a painful stiffening of the shoulder, which he named humero-scapular periarthritis. Codman[2] was the first to coin the term frozen shoulder in 1934, for a condition which was characterized by painful restriction of shoulder motion. Neviaser *et al*[3] suggested the term adhesive capsulitis after a cadaveric study and intra-operative findings of a thickened capsule, adherent to the humeral head. Both terms, frozen shoulder and adhesive capsulitis, are now used interchangeable in the literature for the same condition.

Frozen shoulder affects approximately 2%-4% of the general population[4,5]. The peak incidence is mainly between the age of 40 and 65 years, slightly more frequent in women than in men[6]. The most important associated systemic condition is diabetes mellitus, followed by thyroid disorders. The prevalence of frozen shoulder increases to 10%-20% in diabetic patients. These patients seem to have a prolonged course of the disease, less response to conservative treatment and bilateral involvement is seen more frequently[7,8].

The natural history of a frozen shoulder is described in a relative limited amount of studies. In the majority of patients, it seems to be a self-limited condition with functional recovery after 2-3 years[9]. However, some patients experience continued pain and limited range of motion. After recovery, recurrence of a frozen shoulder is extremely rare[10].

DIAGNOSIS

Although frozen shoulder is a well-known clinical entity, there are still many controversies existing about the definition, the different phases and certainly about the optimal treatment regimen. Zuckerman *et al*[11] proposed this descriptive consensus definition, which was agreed by 82% of members of the American Shoulder and Elbow Surgeons: A condition characterized by functional restriction of both active and passive shoulder motion for which radiographs of the glenohumeral joint are essentially unremarkable except for the presence of osteopenia or calcific tendinitis. Commonly clinical findings consist of: painful stiff shoulder for at least 4 wk; severe shoulder pain that interferes with activities of daily living or work; night pain; painful restriction of both passive and active shoulder range of motion and normal radiographic appearance[12,13]. With physical examination, the selective loss of passive external rotation is typical[14].

Frozen shoulder is usually categorized in primary (or idiopathic) and secondary frozen shoulder. In a primary (idiopathic) frozen shoulder, an underlying aetiology cannot be found. In secondary frozen shoulder, local or intrinsic factors (such as proximal humeral fracture, rotator cuff disorders, biceps tendonitis), remote or extrinsic factors (*e.g.,* ipsilateral breast surgery, cervical radiculopathy, cerebrovascular accident, postoperative immobilization) or systemic pathology (including diabetes mellitus, thyroid disorders, hypo-adrenalism) may be related to the disease[11,12].

In 1975 Reeves[15] believed the condition to involve three separate phases. Phase one, the painful phase followed by phase two, the frozen phase in which pain persists and stiffness is aggravated. Phase three is named the thawing phase, where joint motion and pain gradually improve[15]. A wide variety in the duration of each phase is described, but most authors agree with spontaneous functional recovery after 2-3 years[14,16].

Frozen shoulder is a clinical entity which can generally be diagnosed after a thorough history and physical examination. Plain radiographs are typical without abnormalities. Osteoarthritis of the glenohumeral joint can easily be ruled out. Calcifications in the rotator cuff is a common incidental finding. Ultrasonography is not required for the diagnosis but is appropriate to screen for rotator cuff or biceps tendon abnormalities when suspected. Magnetic resonance imaging (MRI) arthrography can show thickening of the coracohumeral ligament and joint capsule in the rotator interval. Also, synovial thickening in the axillary pouch correlates with the stage of adhesive capsulitis[17]. However, magnetic resonance imaging should not be routinely ordered in the evaluation of the frozen shoulder.

PATHOPHYSIOLOGY

In a secondary frozen shoulder, a local or remote factor that leads to immobilisation of the limb or a systemic condition is an underlying cause to be held accountable for the development of a frozen shoulder. However, most cases of frozen shoulder are primary or idiopathic in which the pathophysiology is not yet fully understood. White *et al*[18] suggest an increase in sedentary jobs with a low level of activity as a possible explanation for the increasing occurrence of a frozen shoulder. A decrease in intra-articular volume and capsular compliance was already described in 1969[19]. An inflammatory contracture of the anterosuperior capsule, the glenohumeral ligaments and the coracohumeral ligament is demonstrated in cadaveric studies and MRI studies[14,20]. This

corresponds with the characteristic clinical finding of loss of external rotation in adduction with physical examination. Significant synovial hypertrophy and neovascular proliferation, especially in the rotator interval is often observed during arthroscopy. A histologic study of Bunker *et al*[21] demonstrates that the predominant cells involved are fibroblasts and myofibroblasts in the joint capsule that produce the extracellular matrix. The produced type Ⅲ collagen matrix is packed more densely, causing the shoulder capsule to contract. This excess of extracellular matrix is characteristic for fibroproliferative disorders. Other histologic changes consist of chronic inflammation and perivascular infiltration and fibrosis[22]. On a cellular level, the extracellular matrix turnover (production, degradation and remodelling) is involved by matrix metalloproteinases and their inhibitors. An imbalance can lead to fibroproliferation, which is demonstrated in frozen shoulder patients[23]. The microscopic changes in the anterior capsule and the coracohumeral ligament are very similar to the changes seen in Dupuytrens' disease in the hand. Dupuytrens' disease is frequently observed in patients with a frozen shoulder[21]. Smith *et al*[24] report an incidence of Dupuytrens' disease of 52% in a cohort of patients with a primary frozen shoulder. Although frozen shoulder has a different natural history than Dupuytrens' disease (self-limiting versus progressive), a common biochemical pathway of both fibroproliferative disorders that leads to contracture is suggested[24].

More recently, the role of inflammatory cytokines and growth factors in the pathogenesis of a frozen shoulder is investigated, because they regulate the growth and function of fibroblasts. The study of Lho *et al*[25] confirmed the overexpression of inflammatory cytokines (such as interleukin 1-α, tumor necrosis factor-α and cyclooxygenase-2) in the joint capsule of patients with a frozen shoulder compared to a control group. Also, an overexpression of these inflammatory mediators was found in tissue samples of the subacromial bursa in frozen shoulder patients[25], possibly contributing to the cascade of inflammation eventually leading to fibrosis.

A future better understanding of the pathophysiology of a frozen shoulder on a cellular level can possibly lead to targeted therapy with anti-inflammatory medication[26].

MANAGEMENT

There are many different strategies in the treatment of a frozen shoulder: including but not limited to supervised neglect[9], physiotherapy[27,28], corticosteroid infiltration[29,30], manipulation under anaesthesia (MUA)[31], arthroscopic capsular release (ACR)[32], arthrographic capsular distension[33] and stretching devices[34]. The optimal treatment regimen has not yet been established. Systematic reviews point to a lack of good quality evidence to give evidence based supported recommendations[35,36]. Non-steroidal anti-inflammatory drugs, intra-articular corticosteroid injections and physiotherapy are among the most widely used treatment modalities in the treatment of a frozen shoulder, in both primary and secondary healthcare settings[35,37]. Because the natural history of a frozen shoulder develops in different phases, it is suggested that the timing of different treatment modalities might be important in this regard. However, there is only a limited amount of good quality studies that have investigated this matter. The positive effect of intra-articular corticosteroid injections appears to be most obvious at an early painful phase of the condition[38,39]. Shin *et al*[40] found a similar positive effect of a subacromial corticosteroid injection compared to an intra-articular injection. The role of physiotherapy is still controversial[41]. Most authors are convinced that the physiotherapy protocol must be adjusted to the phase of the condition with a more important role for physiotherapy in later, less painful phases of the condition. Hanchard *et al*[42] suggest different physiotherapy modalities for a pain-predominant or stiffness-predominant frozen shoulder. Kelley *et al*[43] distinguishes three levels of tissue irritability (high, moderate or low irritability) in frozen shoulder patients to adjust the physiotherapy protocol. Furthermore, other than a primary (idiopathic) frozen shoulder, secondary frozen shoulders after trauma or surgery are often more resistant to conservative treatment[44,45].

Taking above into account, conservative treatment seems to be sufficient for most cases, and almost full recovery takes place in two or three years[14]. Most authors state that failure of at least 6 to 12 mo of appropriate non-operative treatment is an indication for more invasive interventions[46]. However, it is questioned if the course of the disease can be shortened when more invasive interventions are undertaken earlier on in the disease[47]. On the other hand, early surgical intervention for symptomatic frozen shoulder may lead to overtreatment in patients with a mild, self-limiting natural course. It might be interesting to know if it is possible to identify which patients will develop a prolonged course, thus could benefit from early invasive treatment. Prospective studies of non-operative treatment showed that approximately 10% of the patients with an idiopathic frozen shoulder develop a refractory frozen shoulder in which further intervention such as MUA or ACR should be considered[6,9]. MUA is a traditionally well-established technique. However, according to the number of publications on this subject in recent years, ACR is gaining more attention. Both procedures have their own specific advantages and disadvantages.

MVA

The same Duplay[1] who described painful stiffening of the shoulder as humero-scapular periarthritis in 1872 suggested MUA as an appropriate treatment

for frozen shoulder[1]. Before the improvement in arthroscopic shoulder surgery, MUA was the standard treatment of a frozen shoulder if conservative treatment had failed.

Different techniques have been described, but a fixed order of manipulations is recommended. The use of a small lever arm and scapular stabilization is recommended to prevent fractures and brachial plexus traction injuries[48]. First the arm is brought in to full flexion, then cross body adduction followed by external rotation with the elbow adducted against the trunk. Then the arm is abducted and moved into internal and finally external rotation. A characteristic crepitus can be heard and felt by the surgeon as the contracted capsule is ruptured. The addition of an intra-articular injection with corticosteroids and local anaesthetic agent is often used at the end of the procedure.

Consistently satisfactory results in both short- and long-term follow-up are reported with MUA. A significant improvement in range of motion and an overall satisfaction rate of 94% at short term is reported by Dodenhoff *et al*[48]. A major cause of satisfaction was to regain the ability to perform normal daily tasks within days of the manipulation. Long term results confirm that the results do not deteriorate after 15 years[49]. Equal range of motion to the contralateral shoulder and no pain was reported in 90% of the patients after 23 years of follow up in a small cohort[50].

ACR

ACR has gained popularity over the years[51]. The first ACR was described by Conti[52] in 1979. The exact procedure and the magnitude of the capsular release differs between various authors. Earlier techniques describe an anterior and inferior release[46,53]. More recent articles favour a complete circumferential (360 degrees) release[32,54,55].

Both beach chair and the lateral decubitus position with the arm suspended in traction are possible to perform an ACR. However, in the beach chair position it is easier to assess the range of motion of the shoulder during surgery. A pressure pump system and a vasoconstrictive agent (*e.g.,* adrenaline or epinephrine) in the irrigation solution are recommended to improve visibility. The capsular release is performed with a radiofrequency probe. The structures in the rotator interval and the anterior capsule must be released first. Ogilvie-Harris *et al*[46] and Pearsall *et al*[56] recommend to release the intra-articular portion of the subscapularis tendon, however, several studies show excellent results without sacrificing the subscapularis[46,51,55,56]. The superior capsule can be released parallel to the joint surface until the muscular fibres of the supraspinatus are visible. It is also possible to release the posterior inferior aspect of the capsule. However, the benefit of this posterior release could not be confirmed in a recent level 1 randomized controlled trial[57]. A gentle manipulation can be performed to assess the obtained range of motion. Some authors infiltrate the shoulder joint with corticosteroids at this point[54]. Good to excellent results with regard to function and pain at both short and long term after ACR are reported. A large prospective study of smith at all reported good pain relief in 80% of the patients within six weeks[55]. Le Lievre *et al*[54] demonstrated that the obtained improvements of pain and patient reported shoulder function maintained after a mean follow up of seven years. In addition, the shoulder range of motion was comparable with that of the contralateral shoulder at time of follow up.

Postoperative treatment and pain management

Similar rehabilitation protocols after MUA and ACR are described. An important aspect after both MUA and ACR is to start physiotherapy immediately, from day one after the surgical intervention. Postoperative pain management must be adequate to tolerate early physiotherapy treatment. This can be achieved in several ways. Pre-operative regional interscalene block[53], a local intra-articular analgesic injection with or without corticosteroid, an indwelling pain pump in the subacromial space, oral analgetics and icepacks have all been described. Immobilisation in a sling must be discouraged at all times to prevent the shoulder joint from getting stiff again[54]. With adequate pain management, both procedures are assumed to be very well tolerated with minimal postoperative pain[48,51]. Most authors agree on intensive supervised physiotherapy twice or three times a week, possibly supplemented by a home exercise program[53,55].

Pros and cons for manipulation under anaesthesia or arthroscopic capsular release

Comparable satisfactory results are reported by many authors for MUA as well as for ACR. To our knowledge there are no randomized controlled trials comparing manipulation with capsular release for frozen shoulder. A comparison between both procedures was attempted in a recent systematic review primarily based on level Ⅳ evidence. With caution, this study slightly favoured ACR over MUA in recalcitrant idiopathic or diabetic frozen shoulders[12]. The need for prospective higher level evidence is emphasized. The overall complication rate for both procedures is rather low with 0.5% complications reported. The advantages and risks of MUA and ACR are listed in Table 1.

One of the most important arguments used by opponents of MUA, is that it is a fairly uncontrollable procedure. You can not see what is released, or torn within or around the shoulder joint. The potential risks of MUA are wide-ranging. Reported iatrogenic injuries are: proximal humerus or humeral shaft fractures[58], brachial plexus traction injury[59], glenohumeral ligament tears, rotator cuff tears, labral lesions, osteochondral fractures of the anterior glenoid rim[60]. Significant osteopenia can be considered as a relative

Table 1 Advantages and risks of manipulation under anaesthesia and arthroscopic capsular release in the treatment of a frozen shoulder

Advantages	Disadvantages/risks
Manipulation under anaesthesia	
Time efficient	Fracture of humeral shaft or neck
Cost efficient	Rotator cuff tearing
Technical easy procedure	Brachial plexus nerve injury
	Labral lesions
	(Osteo)chondral fracture (glenoid rim)
Arthroscopic capsular release	
Visually controlled capsular release	Less time and cost efficient compared to MUA
Identification and treatment of associated intra-articular pathology	Can be technically more demanding
No excessive bleeding in the joint	Cartilage damage when introducing the arthroscope
	Axillary nerve injury
	Chondrolysis due to heat generation
	Extravasation of fluid in surrounding tissues
	Infection

MUA: Manipulation under anaesthesia.

contra indication to MUA. Although a lot of articles address the risk of a humeral fracture and the use of a short lever arm is emphasized, the complication itself is seldom reported[58,61]. Loew *et al*[60] performed an arthroscopy directly after MUA in 30 persons to investigate the intra-articular damage. Hemarthrosis was found in all patients. The anterior capsule was ruptured in 22 out of 30 shoulders, mostly adjacent to and parallel to the labrum, where it is intended to tear. Unequivocal lesions were found in 12 out of 30 shoulders, this involved the anterior and superior labrum, partial tears of the subscapularis tendon, the supraspinatus tendon, the long head of the biceps and one small osteochondral fracture[60]. An evident advantage of MUA in comparison to ACR is that it is more time efficient and that it is associated with substantial lower costs.

Proponents of the ACR procedure believe that a complete release of the capsule can be achieved in a more controlled way. Associated intra-articular pathology can be identified and treated simultaneous. The risks are fairly low, with a documented complication rate of 0.5%[12,45]. However, serious complications as axillary nerve injury, chondrolysis and skin burns due to heat generation or infection are documented[3,45,62]. Nowadays, temperature controlled diathermal probes are commercially available, possibly preventing overheating of the fluids in the joint during surgery. Different from MUA, ACR can be a more technical demanding procedure. Some authors even state that ACR should only be done when MUA has failed[14].

Another option is to combine ACR with manipulation. The manipulation can be a gentle one only to release the capsule where it is difficult to reach or risky to release arthroscopically (for example in the area of the axillary nerve). Early significant improvement in shoulder range of motion with relief of pain and maintenance of these results at long term are reported[41,54,55].

CONCLUSION

A frozen shoulder is a common cause of shoulder pain and stiffness. The majority of the patients can be treated conservatively, with functional recovery to be expected after two to three years. However, if conservative treatment fails, MUA and ACR can both be considered as appropriate treatments. MUA is an easy, time- and cost-efficient technique, but is accompanied by the risk of iatrogenic damage. ACR seems to be a safer way to release the joint capsule. Associated intra-articular pathology can be identified and bleeding can be controlled. However, ACR is technically more demanding, and is also accompanied by the risk of damage to the cartilage or the axillary nerve. Both procedures are performed in large numbers and are considered safe and beneficial for the patient. Superiority of one technique over the other can't be supported by randomized trials comparing both techniques. In addition, the optimal timing of any surgical intervention for frozen shoulder has to be determined yet. Therefore, the decision for either one procedure to treat a frozen shoulder is made by the orthopaedic surgeon and the individual patient together.

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EDITORIAL

Unsaturation index and type 2 diabetes: Unknown, unloved

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Abstract

A useful parameter for interpreting analyses of membrane fatty-acid composition is the unsaturation index (UI), a measure of unsaturation that is calculated as the mean number of cis double bonds per fatty-acid residue multiplied by 100. The UI is a fundamental parameter that contains information about many membrane biophysical properties and behavior. UI is a crucial index for type 2 diabetes (T2D) and other disorders, yet it is not properly considered in the scientific community. The goal of the present editorial is to familiarize the scientific T2D community with the UI. The idea of early systemic cell-membrane disease necessitates new thinking and suggests that UI should feature prominently on the research agenda.

Key words: Type 2 diabetes; Unsaturation index; Phospholipid; Cell membrane; Fatty acid

Core tip: A useful parameter for interpreting analyses of membrane fatty-acid composition is the unsaturation index (UI), a measure of unsaturation that is calculated as the mean number of *cis* double bonds per fatty-acid residue multiplied by 100. The UI is a fundamental parameter that contains information about many membrane biophysical properties and behaviour. UI is a crucial index for type 2 diabetes (T2D) and other disorders, yet it is not properly considered in the scientific community. The goal of the present editorial is to familiarize the scientific T2D community with the UI.

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INTRODUCTION

A useful parameter for interpreting analyses of membrane fatty-acid composition is the unsaturation index (UI), a measure of unsaturation that is calculated as the mean number of *cis* double bonds per fatty-acid residue multiplied by 100[1]. This parameter characterizes a phospholipid bilayer and describes the fluidity, or flexibility, of a biological membrane. As the UI increases, so does the distance between plasma-membrane fatty-acyl chains, decreasing their mutual attraction energy and thus increasing membrane flexibility, which promotes an increase in the number of all functional Class I glucose transporters per

Table 1 Unsaturation index of erythrocyte membrane fatty-acid composition of controls and type 2 diabetes patients without diabetic retinopathy

Fatty acids	Controls (n = 18)		T2D patients without diabetic retinopathy (n = 14)	
	% of total fatty acids[1]	× number of double bonds	% of total fatty acids[1]	× number of double bonds
C14:0	0.43	-	0.48	-
C15:0	0.17	-	0.22	-
C16:0	22.41	-	23.75	-
C17:0	0.38	-	0.44	-
C18:0	17.22	-	17.72	-
C20:0	0.15	-	0.18	-
C22:0	0.33	-	0.42	-
C24:0	0.74	-	0.78	-
C16:1	0.73	0.73	1.03	1.03
C18:1 (trans)	0.22	-	0.2	-
C18:1	17.16	17.16	19.15	19.15
C20:1 n-9	0.21	0.21	0.26	0.26
C20:3 n-9	0.19	0.57	0.2	0.6
C22:1 n-9	0.06	0.06	0.09	0.09
C24:1 n-9	0.69	0.69	1.15	1.15
C18:2 n-6	12.87	25.74	10.58	21.16
C18:3 n-6	0.09	0.27	0.12	0.36
C20:2 n-6	0.2	0.4	0.23	0.46
C20:3 n-6	1.3	3.9	1.5	4.5
C20:4 n-6	13.04	52.16	11.33	45.32
C22:4 n-6	2.0	8	2.01	8.04
C22:5 n-6	0.35	1.75	0.3	1.5
C18:3 n-3	0.22	0.66	0.21	0.63
C20:5 n-3	0.97	4.85	1.03	5.15
C22:5 n-3	2.23	11.15	1.56	7.8
C22:6 n-3	4.51	27.06	2.85	17.1
Unsaturation index[2]		155.36		134.3

[1]Data published by Koehrer *et al*[5]; [2]The unsaturation index was calculated as the mean number of double bonds per fatty acid residue multiplied by 100.

membrane surface area[2]. At the most basic level, the basal metabolic rate of a cell is directly linked to its cell membrane acyl composition, and thus to its UI[3]. To date, this relationship has not received due attention in the treatment for type 2 diabetes (T2D).

The UI is a fundamental parameter that contains information about many membrane biophysical properties and behavior. Arachidonic acid and docosahexaenoic acid are key fatty acids; a minimal increase in the percentage of arachidonic acid in phospholipid tails improves membrane flexibility due to its four double bonds. A similar effect is seen for docosahexaenoic acid, with its six unsaturated bonds. UI is a crucial index for T2D and other disorders, yet it is not properly considered in the scientific community[4]. The goal of the present editorial is to familiarize the scientific T2D community with the UI.

In the September issue of *PLoS ONE*, Koehrer *et al*[5] reported the erythrocyte phospholipid and polyunsaturated fatty-acid composition in diabetic retinopathy. Several points in this article require additional clarification. Given that the study consisted nearly exclusively T2D patients, the reported observations are likely to be restricted to this type of diabetes. In contrast to one previous publication, Koehrer *et al*[5] presented measurements of total phospholipids from red blood cells, with fatty-acid composition specified for a total of 26 fatty acids. Based on the presented data, we calculated the UIs of membrane phospholipids from control subjects, T2D patients with and without retinopathy, and patients with gestational diabetes mellitus[6]. For example, Table 1 describes the calculation of the UIs for controls and T2D patients without retinopathy included in the study of Koehrer *et al*[5].

The UIs based on the erythrocyte membrane fatty-acid compositions reported in these studies[5,6] yielded novel information (Table 2). First, although phosphatidylcholine and phosphatidylethanolamine comprise about 60% of the total phospholipid in the bilayer membrane of human erythrocytes, the red cell phosphatidylcholine and phosphatidylethanolamine UI of subjects with normal glucose tolerance in the gestational diabetes mellitus study[6] are in line with the total phospholipid UI of the reference population in the diabetic retinopathy study[5] (162.8 and 155.4, respectively; Δ = 4.5%). Second, the decrease in the UI of phosphatidylcholine and phosphatidylethanolamine for gestational diabetes mellitus patients relative to controls was substantially higher than the total phospholipid UI decrease for T2D individuals without diabetic retinopathy compared with controls (16.3% and 13.5%, respectively; Δ = 17.2%), due to two underlying phenomena, *i.e.,* a temporary gestational and a chronic prediabetic increase in plasma FFA[7]. Third, the total phospholipid UI was substantially lower in T2D individuals than in healthy controls (134.3 and 155.4, respectively; Δ = 13.5%). Finally, the mean total phospholipid UI was substantially lower in T2D individuals with mild, moderate, and severe diabetic retinopathy than in T2D individuals without diabetic retinopathy (123.4 and 134.3, respectively; Δ = 8.1%). These experimental outcomes indicate that membrane flexibility plays an important role in microvascular complications of T2D. Further, these data support our working hypothesis: a gradual elevation of the plasma levels of saturated and monounsaturated free fatty acids causes a decrease in the number of polyunsaturated fatty-acyl chains in membrane phospholipids[2], a classical principle of membrane biogenesis[3,8]. In this context, it is noteworthy that our working hypothesis predicts that the transition from a healthy condition to a state with T2D will be matched by a decrease in UI, as will the transition from T2D without diabetic retinopathy to T2D with retinopathy[2].

In a study of the relationship between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids, Borkman *et al*[9] concluded that reduced levels of unsaturated fatty acids in the membrane may be due to a net reduction in the action of insulin, as a consequence of either insulin resistance or insulin deficiency or, alternatively, as a consequence

Table 2 Calculated unsaturation indices based on erythrocyte fatty-acid compositions reported by several studies

Disease	Participants (*n*)		Erythrocyte membrane	Unsaturation index[1]		Decrease (%)	Ref.
	Controls	Disease		Controls	Diabetic subjects		
T2D/T1D	18	13/1	Total phospholipid	155.4	134.3	13.5	[5]
T2D/T1D + mild DR	18	11/1	Total phospholipid	155.4	125.9	19.0	[5]
T2D/T1D + moderate DR	18	11/1	Total phospholipid	155.4	119.5	23.1	[5]
T2D/T1D + severe DR	18	19/3	Total phospholipid	155.4	124.7	19.7	[5]
T2D/T1D + proliferative DR	18	17/7	Total phospholipid	155.4	136.9	11.9	[5]
Gestational diabetes	61	53	PC + PE	162.8	137.1	16.3	[6]

[1]The unsaturation index was calculated as the mean number of double bonds per fatty acid residue multiplied by 100[4]. DR: Diabetic retinopathy; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine.

of hyperinsulinaemia. This interpretation seems unlikely for the following reasons: first, gestational diabetes mellitus is a marker of a prediabetic phase characterized by time-dependent increase in insulin levels[10,11], where the T2D phase is marked by a time-dependent decrease in insulin levels[11]. Second, we demonstrated that patients in both phases were associated with lower UIs than were healthy controls, which suggests that insulin levels do not have an important causative role in lowering the UI. We hypothesize that a gradual increase in plasma free fatty-acid concentration during the prediabetic phase and after overt T2D[12,13] decreases the UI[7].

A well-known characteristic of the euglycaemic hyperinsulinaemic clamp is its wide inter-subject variability in insulin sensitivity. In a study of metabolic effects of lacidipine: a placebo-controlled study using the euglycaemic hyperinsulinaemic clamp, Morris *et al*[14] reported that even amongst non-diabetic subjects who were homogeneous for age, sex and body weight there was a wide inter-subject variability in insulin sensitivity, *i.e.*, 5.6-16.2 mg/kg per minute where the intra-subject variability in insulin sensitivity on the two placebo study days was 9%. Since physical activity and caloric intake are individual entities, which significantly affect a persons' free fatty acid concentration, we suggest that the wide inter-subject variability may be attributable to the inter-subject variability in free fatty acid concentration, and thus in the individual UI[13].

Despite extensive guidelines for managing T2D, in the United States during the years 2005-2008, 28.5% of adults with diabetes aged 40 years or older had diabetic retinopathy and 4.4% had advanced diabetic retinopathy[15]. These incidences are probably due to a longstanding period of decreased UI, increasing the stiffness of both the erythrocyte and plasma membranes and, as a consequence, decreasing microcirculatory flow, ultimately leading to chronic tissue hypoxia, insufficient tissue nutrition, and diabetes-specific microvascular pathology[2]. Thus, the idea of early systemic cell-membrane disease necessitates new thinking and suggests that UI should feature prominently on the research agenda.

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EDITORIAL

Low back pain-related meta-analysis: Caution is needed when interpreting published research results

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Abstract

The systematic reviews (SRs) including a meta-analysis are considered as the top level of evidence. Although the existence of more than a hundred of low back pain (LBP)-related SRs seems very appealing and might therefore suggest significant evidence on the topic, a deep analysis indicates that several of these SRs included only very few studies. Other SRs raise concerns because they included some randomized controlled trials which had a low methodological quality, or some studies which differed significantly regarding the studied populations and/or the experimental procedure. The sometimes controversial results of different SRs conducted on the same topic also highlight the significant influence of the inclusion/exclusion criteria used in the SRs to select the articles. To conclude, although meta-analysis is at the top of the evidence pyramid and have several strengths, the conclusions drawn from SRs should always be interpreted with caution because they can also have weaknesses. This is true, whether it be for LBP-related SRs including a meta-analysis, or any other. Therefore a critical analysis of any SR is always needed before integrating the results of the SR in its own clinical practice. Furthermore, clinical reasoning remains crucial, especially to consider the potential differences between one's patient and the patients included in the meta-analysis.

Key words: Meta-analysis; Systematic review; Spine; Back pain; Limitations; Recommendations; Evidence-based practice

Core tip: Although meta-analysis are at the top of the evidence pyramid and have several strengths, the conclusions drawn from systematic reviews combined to a meta-analysis should always be interpreted with caution because they can also have weaknesses. This is true, whether it be for low back pain-related systematic reviews including a meta-analysis, or any other. Therefore, a critical analysis of a systematic review is always needed before integrating the results in its own clinical practice. Furthermore, clinical reasoning remains crucial, especially to consider the potential differences between one's patient and the patients included in the

meta-analysis.

Demoulin C, Bruyère O, Somville PR, Vanderthommen M. Low back pain-related meta-analysis: Caution is needed when interpreting published research results. *World J Meta-Anal* 2015; 3(2): 93-96 Available from: URL: http://www.wjgnet.com/2308-3840/full/v3/i2/93.htm DOI: http://dx.doi.org/10.13105/wjma.v3.i2.93

INTRODUCTION

Nowadays, the relevance and necessity to treat patients according to the evidence-based medicine is widely recognized[1]. This approach aims to integrate clinical expertise, patient values and the best research evidence[1,2]. Regarding the latter component, it has become impossible for clinicians, and even scientists, to read all the papers on a specific topic considering the constant increasing number of scientific studies conducted every year[3]. Therefore, narrative and systematic reviews (SRs) have become very popular "easy access" methods for clinicians and researchers to help them to overview the scientific literature[1]. In contrast to the narrative reviews, which are rather qualitative summaries based on the knowledge of an experienced author, the SRs are performed according to a complex but very transparent process of search[4]. Furthermore, its combination with a meta-analysis provides a statistical summary of the evidence (*e.g.,* treatment effect, diagnostic method, prognosis, *etc.*) regarding a given topic, thereby facilitating the integration of the best evidence into practice. Meta-analysis also allow increasing the power, improving precision, and analyzing the consistency of effects[4,5]. Because the systematic reviews with a meta-analysis are considered as the top level of evidence, their results can greatly influence the guidelines and decisions related to a specific topic.

The number of meta-analysis published in the scientific literature is increasing in an exponential way[6]; the low back pain (LBP) field is no exception. This is well illustrated by a quick search of Pubmed with the generic terms "low back pain" and "meta-analysis" at the beginning of January 2015 which resulted in 377 hits, among which half of them (*n* = 166) were published over the last five years. An analysis of the hits reveals that two thirds of these 166 hits were effectively SRs combined to a meta-analysis related to LBP. The main topics concerned surgery, exercises, injections, pharmacological treatments, risk factors, spinal manual/manipulative therapies and imaging. Most of them were meta-analyses of randomized controlled trials (RCTs). There were also an overview of systematic reviews[7] and a SR of systematic reviews[8] reflecting the expansion of the SRs.

Although the existence of more than a hundred of LBP-related SRs seems very appealing and might therefore suggest significant evidence on the topic, a deep analysis indicates that several of these SRs combined to a meta-analysis included only very few studies (because of the low number of studies on the topic and/or of the low methodological quality of several studies) (*e.g.,*[9,10]). In some SRs, a meta-analysis was planned but was not conducted due to a lack of articles on the topic (*e.g.,*[11]) or a high clinical heterogeneity between studies regarding the intervention (*e.g.,*[12,13]) or the functional outcome measures (*e.g.,*[14]). The choice to conduct or not a meta-analysis appears subjective and differed between authors (*e.g.,* Hansen *et al*[11] decided to perform it only if the literature search resulted in at least 5 RCTs meeting the predefined inclusion criteria and if they were homogeneous whereas other performed a meta-analysis based only on three studies (*e.g.,*[10]).

Other SRs raise concerns because they included some RCTs which had a low methodological quality (*e.g.,* 5 out of the 7 RCTs included in the review of Ebadi *et al*[15] on the effectiveness of therapeutic ultrasound for chronic LBP had a score < 6/12 when using the 12 criteria recommended by the Cochrane Back Review Group) or some studies which differed significantly regarding the experimental procedure (*e.g.,* treatment provided). The pooling of spinal mobilizations and spinal manipulations[16], which are two different techniques regarding the indications, effects, *etc.*, or of a specific technique (manipulation) and a manual therapy concept (combination of techniques)[17], are good examples of mixing heterogeneous studies[18]. Another example of not ideal pooling is taken from meta-analysis regarding the effectiveness of "exercises" for LBP. Indeed, some conducted a calculation of a summary estimate[19] although there exists so many different types of exercise and although some parameters might influence the treatment outcomes[20] (*e.g.,* the number of sessions[21]). Another review on the topic tried to distinguish the different types of exercises[22], but the pooling was not always relevant[23]. Thus, reviewing the effectiveness of some LBP-related treatments (*e.g.,* physical therapy) is much more complex than for other treatments (*e.g.,* drug therapy) which are less heterogeneous between studies.

The characteristics of the participants are other crucial parameters to consider when conducting and interpreting a SR related to LBP. Indeed, in contrast to specific diseases (*e.g.,* influenza), "non-specific LBP" has no identifiable cause and is rather a symptom. Furthermore, according to the bio-psycho-social model and the literature on the topic, numerous (individual, psychosocial, work-related) factors influence the outcome/prognosis of musculoskeletal pain and should be taken into account when treating a patient, especially in case of chronic pain[24,25]. Therefore, lots of subgroups of patients with LBP have been described in literature[26]. A meta-analysis about the effectiveness of classification-based interventions reveals that such interventions

seem more effective for reducing pain and disability than "standard" treatments. This highlights the potential bias when pooling studies with different populations. To solve this problem, a subgroup analysis can be conducted to study the influence of several parameters. However, one has to keep in mind that an effect of chance can occur when analyzing too many subgroups[27]; moreover, a low number of studies included in the SRs prevents to achieve such an analysis.

The inclusion/exclusion criteria used in the SRs to select the articles are also important to consider when interpreting the results of a meta-analysis because they can have a significant influence[6]. Indeed, a SR of the SRs conducted on the effectiveness of the Pilates exercises in patients with chronic LBP revealed that the 5 SRs which had been published on the topic by that time had different conclusions although they had similar research objectives[8]; furthermore, only two out of the ten primary studies were included in the 5 papers[8]. Besides, some SRs considered only quantitative results from questionnaires while ignoring qualitative studies which might therefore introduced also a bias[28].

Another point to be noticed about the LBP-related meta-analyses is that most of them have been conducted on aggregate-level data whereas only a few have been performed on individual data (*e.g.,*[29]), although the latter analysis appears very relevant[30].

To conclude, although meta-analyses are at the top of the evidence pyramid and have several strengths, the conclusions drawn from SRs should always be interpreted with caution because they can also have weaknesses. This is true, whether it be for LBP-related SRs including a meta-analysis, or any other. Interpreting results of a meta-analysis is not easy, as evidenced by the letters to the editor[18,31] related to the SR of Licciardone *et al*[17]. Using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis checklist can help readers for critical appraisal purposes. Besides, analyzing the methodology of the SR, examining the studies included (*e.g.,* population, intervention, outcome) and thinking about the possible sources of heterogeneity of the results (in case it occurs) are really necessary before integrating the results of the SR in its own clinical practice[2,6,27]. Furthermore, clinical reasoning remains crucial[32], especially to consider the potential differences between one's patient and the patients included in the meta-analysis[27]. Only reading the abstract of a SR combined to a meta-analysis is clearly not good enough to do so.

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SYSTEMATIC REVIEWS

Meta-analysis of single strain probiotics for the eradication of *Helicobacter pylori* and prevention of adverse events

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Author contributions: McFarland LV, Malfertheiner P, Huang Y and Wang L reviewed, scored papers and were involved in the manuscript preparation; McFarland LV designed the study, did literature search and analyzed the data.
Conflict-of-interest: Lynne V McFarland has received fees as a speaker (Biocodex, France and Lallemand, France) and is on the scientific advisory board of BioK+, Canada. Peter Malfertheiner has received speaker fees from Biocodex and is on the scientific advisor board of Danone. Ying Huang and Lin Wang have no conflicts of interest. No authors are employed by or have stock or equity in any company manufacturing probiotics.
Data sharing: No additional data are available.

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Abstract

AIM: To assess the efficacy and safety of single strain probiotics for the: (1) eradication of *Helicobacter pylori* (*H. pylori*); (2) prevention of adverse events; and (3) prevention of antibiotic-associated diarrhea associated with eradication therapy.

METHODS: We searched PubMed (1960-2014), EMBASE (1974-2014), Cochrane Database of Systematic Reviews (1990-2014), and ISI Web of Science (2000-2014). Additionally, we conducted a grey literature search including contact with National Institutes of Health Clinical Trials Registry, abstracts from annual infectious disease and gastroenterology meetings, experts in the field and correspondence with authors. Randomized controlled trials of *H. pylori* positive adults or children treated with eradication therapy and assessing the adjunctive therapy with a single strain of probiotics were included. The primary outcomes were the rates of eradication of *H. pylori* and frequency of patients with adverse events or antibiotic-associated diarrhea. Outcomes were pooled using fixed or random-effects models to calculate the relative risk and corresponding 95%CI and weighted on study size. To explore possible explanations for heterogeneity, a priori subgroup analyses were conducted on daily probiotic dose, study population, and quality of the study. The overall quality of the evidence for each probiotic strain was assessed using the GRADE criteria.

RESULTS: A total of 25 randomized controlled trials (28 treatment arms, with a total of 3769 participants) assessed one of six single probiotic strains as adjunctive treatments to standard eradication therapy. Only one probiotic strain significantly improved *H. pylori* eradication rates: *Saccharomyces boulardii* (*S. boulardii*) CNCM I-745 [pooled relative risks (pRR) = 1.11, 95%CI: 1.07-1.16]. Only one probiotic strain (*S. boulardii* CNCM I-745) significantly prevented any adverse events (pRR = 0.42, 95%CI: 0.28-0.62). Both *S. boulardii* CNCM I-745 and *Lactobacillus rhamnosus* GG significantly

reduced antibiotic-associated diarrhea (pRR = 0.47, 95%CI: 0.37-0.60 and pRR = 0.29, 95%CI: 0.17-0.48, respectively) associated with *H. pylori* eradication therapy. Meta-regression of sub-groups did not detect significant differences by dose, adult *vs* pediatric, symptom status, or study quality, but did find significant differences by the strain of probiotic. Potential mild publication bias was found for antibiotic-associated diarrhea, but not for eradication or adverse event outcomes. Analysis of the study quality illuminated areas for improvement in future studies (use of placebos, study size calculations, attrition reasons and discussion of limitations and generalizability).

CONCLUSION: The pooled evidence suggests that the adjunctive use of a few probiotic strains may improve *H. pylori* eradication rates and prevent the development of adverse events and antibiotic-associated diarrhea in those treated with standard eradication therapies. The type of probiotic strain was the most important factor in predicting efficacy.

Key words: Probiotics; Safety; *Saccharomyces boulardii*; *Helicobacter pylori*; Meta-analysis; Adverse reactions; Diarrhea; *Lactobacillus rhamnosus*; Randomized clinical trials

Core tip: A meta-analysis was conducted (1960-2014) for randomized clinical trials testing single strained probiotics as an adjunct to standard *Helicobacter pylori* (*H. pylori*) eradication therapy. Of the single strains with multiple trials, only one significantly improved *H. pylori* eradication rates {*Saccharomyces boulardii* (*S. boulardii*) I-745 [pooled relative risks (pRR) = 1.11, 95%CI: 1.07-1.16)]}, while two strains significantly reduced the rate of antibiotic-associated diarrhea [*S. boulardii* I-745 (pRR = 0.47, 95%CI: 0.37-0.60) and *Lactobacillus rhamnosus* GG (pRR = 0.29, 95%CI: 0.17-0.48)]. None of the other four probiotic strains improved *H. pylori* therapy (*C. butyricum*, *L. acidophilus*, *L. reuteri*, *L. casei*).

McFarland LV, Malfertheiner P, Huang Y, Wang L. Meta-analysis of single strain probiotics for the eradication of *Helicobacter pylori* and prevention of adverse events. *World J Meta-Anal* 2015; 3(2): 97-117 Available from: URL: http://www.wjgnet.com/2308-3840/full/v3/i2/97.htm DOI: http://dx.doi.org/10.13105/wjma.v3.i2.97

INTRODUCTION

Helicobacter pylori (H. pylori) was first associated with chronic gastritis, duodenal and peptic ulcers by Marshall and Warren[1] in 1984. Surveillance studies since that time have found *H. pylori* colonization is a global concern with a prevalence ranging from 70%-90% in developing countries and 25%-50% in developed countries[2]. *H. pylori* is typically acquired during childhood from other humans and transmitted by the oral-oral or oral-fecal route or by ingestion of contaminated water. *H. pylori* infection in childhood may lead to chronic gastritis, but only 20% will develop clinical symptoms[2]. Prolonged carriage may result in an onset of symptoms in adults, which include dyspepsia, peptic or duodenal ulcers, gastric adenocarcinoma, B-cell lymphoma and rarely extragastric complications[3]. Current guidelines from the Maastricht Ⅳ consensus for the eradication of *H. pylori* include triple therapy [typically two antibiotics and a proton-pump inhibitor (PPI) for 7-14 d], with eradication rates ranging from 71% to 81%, sequential therapy (with slightly improved *H. pylori* eradication rates from 85% to 84%) and, more recently, bismuth-based quadruple therapy (with 90% efficacy)[4-7]. However, the common development of adverse events [such as antibiotic-associated diarrhea (AAD), nausea, *etc.*] from the eradication therapies cause many patients to prematurely discontinue their treatments, leading to plummeting eradication rates and the development of antibiotic resistance[8-10]. The development of antibiotic resistant strains of *H. pylori* varies by country and type of antibiotic exposure (ranging 11%-29% for clarithromycin, 17%-86% for metronidazole, levofloxacin 14%-24%)[11,12]. In addition, relapses of symptoms occur > 40% in patients within 32 wk after triple therapy eradication therapy[13]. Recently several alternative treatments, including probiotics, have been tested to improve eradication rates, prevent the development of antibiotic resistant strains and to prevent the development of adverse events[14].

Probiotics (defined as living microbes, given in adequate doses, with proven health effects) have been shown to be effective in many diseases and may be useful as an adjunct to eradication therapy. Probiotics are known to be effective for the prevention of side-effects of antibiotic use, typically antibiotic-associated diarrhea[15]. Several studies have also shown some probiotic strains [*Saccharomyces boulardii* (*S. boulardii*), *Lactobacillus acidophilus* (*L. acidophilus*) or mixtures of strains, *etc.*] have specific mechanisms of action against *H. pylori,* including inhibiting *H. pylori* attachment to mucosal cells[16-18], regulation of the immune response to *H. pylori*[19], or direct physiologic effects[20]. Probiotics may also restore the normal microbiota disrupted by antibiotic exposure (causing diarrhea or colitis) and thus prevent *H. pylori*-associated adverse events[21,22].

Choosing the appropriate probiotic can be challenging as the choice must be matched to both probiotic strain and the disease being treated (or prevented), based on the strength of evidence-based clinical trials. Different mechanisms of action are strain-specific, therefore it is necessary to analyze the efficacy by similar probiotic strains whenever possible[23-25]. Most meta-analyses of probiotics for *H. pylori* infections have not done this. Probiotics are also available as single strain products or in mixtures of two or more probiotic strains. This paper will focus only on single strains tested in at least two

randomized, controlled trials.

The aims of this meta-analysis are to analyze the effectiveness of adjunctive single strain probiotics for the: (1) eradication of *H. pylori*; (2) reduction of adverse events; and (3) reduction of antibiotic-associated diarrhea commonly linked with eradication therapy.

MATERIALS AND METHODS

Study objectives

Primary aims: (1) To systematically assess whether single strain probiotics (given as an adjunct with *H. pylori* eradication therapy) could improve the eradication rate of *H. pylori*; and to systematically assess whether probiotics could reduce the frequency of: (2) any types of adverse events; or (3) antibiotic-associated diarrhea associated with *H. pylori* eradication therapy.

Secondary aims: To systematically assess if differences in effect were associated with specific subgroups, defined by: daily dose effect of probiotics, type of study population (adult versus pediatric, asymptomatic versus symptomatic), study quality and strain of probiotic used.

Search strategy

As shown in Table 1, this meta-analysis followed PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analysis) statement guidelines[26] and guidelines using clearly delineated parameters, a *priori* inclusion and exclusion criteria and standardized data extraction tools[27,28]. We undertook systematic searches of PubMed (1960-2014), EMBASE (1974-2014), Cochrane Database of Systematic Reviews (1990-2014), ISI Web of Science (2000-2014) and three on-line clinical trial registries: Cochrane Central Register of Controlled trials (http://www.cochrane.org), MetaRegister of Controlled Trials (http:www.controlled-trials.com/mrct) and National Institutes of Health (http://www.clinicaltrials.gov). We used bibliographies of all relevant studies to do a recursive search. Additionally, we conducted an extensive grey literature search including abstracts from annual infectious disease and gastroenterology meetings, probiotic product websites, experts in the field and communication with published authors on *H. pylori* infections. Search terms included: *H. pylori*, randomized controlled trial and probiotics and specific probiotic strains. Search strategies were broad-based initially, then narrowed to the disease and population of interest. Abstracts of all citations and retrieved studies were reviewed and rated for inclusion. Full articles were retrieved if probiotics were given to treat *H. pylori* infections or carriage or to prevent adverse events associated with *H. pylori* eradication therapies.

Inclusion and exclusion criteria

Inclusion criteria included randomized (well described or partially) controlled trials (RCT), blinded or open trials, in pediatric or adult populations (inpatient or outpatients), published in peer-reviewed journals or on clinical trial websites, or as meeting abstracts. All participants were required to have received *H. pylori* eradication therapy (double, triple, quadruple or sequential therapy) that included at least one antibiotic and one PPI. Non-English language trials were translated and included whenever possible. Exclusion criteria included pre-clinical studies, safety, kinetic or formulation phase 2 studies, case reports or case series, duplicate reports, trials of unspecified types of probiotics, non-randomized trials, incomplete or no outcomes reported, or if translation could not be obtained. Trials which did not assess either *H. pylori* eradication rates or the incidence of adverse events were excluded. Probiotic strains with only one randomized controlled trial (lacking at least one other confirmatory trial) were also excluded. Randomized controlled trials testing probiotic products with a mixture of different probiotic strains were reviewed, but will be presented elsewhere.

Data extraction

Each article was reviewed and scored independently by at least two reviewers. One reviewer (LVM) screened all abstracts, extracted and scored all articles using pre-constructed and piloted, data extraction forms (see Figure 1). Each of three other reviewers (PM, YH, LW) independently extracted data and assessed risk of bias from one-third of the articles (each sent different articles). Any disagreements were resolved by a third reviewer. For articles published in abstract form only or for any missing significant data in full articles, further information was sought by contacting authors or by the company manufacturing the probiotic product. Using a standardized data extraction form, we systematically collected the following data: authors, year of publication and journal, population data (age range, setting, types of eradication therapy given), study aims and outcomes, study methods (study design, eligibility criteria, sample size calculations, interim analysis, statistical methods used, recruitment methods, subgroup analysis done), randomization (method of randomization allocation, randomization method), degree of blinding (open, single or double), intervention data (probiotic strains used, daily dose, duration of treatment, duration of follow-up, type of control used, treatment concealment), results (balanced randomization achieved, attrition rate and reasons, comparison of treatment groups by demographics, *etc.*, CONSORT flow-chart provided), outcome data [by group, intent-to-treat (ITT) or as-per-protocol (APP) analysis], safety data (adverse events reported by group), discussion points (limitations, generalizability and comparison of study results to published papers), clinical trial registration, location of protocol, and

Table 1 Preferred reporting items for systematic reviews and meta-analyses checklist 2009[26]

Item	Topic	Reported on page
Title		
1	Title includes systematic review or meta-analysis or both	97
Abstract		
2	Structured abstract/summary background, objectives, data sources, eligibility criteria, participant, interventions, appraisal and synthesis methods, results, limitation, conclusions and implication of key findings, systematic review registration number	97
Introduction		
3	Rationale for review, what is already known	98
4	Objectives: Specific questions addressed: (PICOS)-participants, interventions, comparisons, outcomes, study design	99
Methods		
5	If review protocol (location and accessed URL, registration number)	NA
6	Eligibility criteria (study characteristics (PICOS, follow-up, *etc*.) and report characteristics (years searched, language, publication status), provide rationale	99
7	Information sources (databases with dates of coverage, contact with study authors to identify additional studies, date last searched)	99
8	Search strategy: Full search strategy for at least one database, including any limits used, such that it could be repeated	99
9	Study selection: (process for screening, eligibility)	99
10	Data collection process: Method of data extraction (piloted forms, independently, in duplicate) and any processes of obtaining and confirming data from investigators)	99
11	Data items: List and define all variables sought (*e.g*., PICOS, funding sources, *etc*.) and any assumptions	99-102
12	Risk of bias in individual studies: (Describe methods used for assessing risk of bias (at study or outcome level), how this info is to be used in any data synthesis)	102
13	Summary Measures: State principal summary outcome measures (RR or Difference in means) for pooled estimates of risk	103
14	Synthesis of results: Describe method of handling data and pooling data (measures of consistency with I2 for each meta-analysis)	103
15	Risk for bias across studies: (publication bias)	103
16	Additional analysis: Any subgroup or sensitivity analysis, meta-regression and if pre-specified	103
Results		
17	Study selection: N of RCT screened, # assessed for eligibility, reasons for exclusions, with flow diagram	103-104, Figure 2
18	Study characteristics (for each study: study size, PICOS, follow-up with citations)	Table 4
19	Risk of bias within studies: Data on risk for bias and if there, any outcome level assessment (see #12, study quality)	107
20	Results of individual studies: Simple summary data for txt arm, effect estimates and confidence intervals for each study, with forest plot	Figures 3-5 Tables 4, 5
21	Synthesis of results: Data on each meta-analysis, pooled data, 95%CI and measures of consistency	Figures 3-5
22	Risk of bias across studies: results of any assessment of risk across studies (see #15)	Figures 6-8
23	Additional analysis data: if done (see #16, sub-groups)	107
Discussion		
24	Summary of evidence: Summarize main findings, strength of evidence for each main outcome. Relevance to key groups (providers, users, policy makers)	110
25	Limitations: Limitations at study level and outcome level (risk of bias), at review-level (incomplete retrieval of identified research, reporting bias)	113
26	Conclusions: General interpretation of results compared to other evidence, implications for future research.	113
Funding		
27	Funding: describe funding sources	Not found

The PRISMA Statement. Available from: URL: http://prisma-statement.org/statement.htm. Accessed 7/25/2014.

source of funding.

Interventions

Included trials had participants who were randomized to either an adjunctive probiotic group or a control group. The type of control group may have included either a placebo (blinded study) or no treatment (open study) in addition to the eradication therapy currently used as standard practice. The type of probiotic intervention included probiotics in any form (*e.g.*, capsule, sachet, tablets, drink, *etc.*) given in conjunction with the *H. pylori* eradication therapy. Trials investigating non-specific probiotics or yogurts [*e.g.*, articles not providing the probiotic strain(s) used] were excluded. Trials combining probiotics with prebiotics were included if the prebiotic dose was less than 2.5 g/d, as this was judged to be of limited impact to alter the intestinal microflora[29,30]. The most recent probiotic strain designations are presented in this study for those strains whose names have changed over time (older articles may have reported a different strain designation). The taxonomy of the probiotic strain type was confirmed by correspondence with authors or the manufacturing companies.

Reference:

First reviewer: _____ Second reviewer: _____

Study design (methodological)

____ 1. □ Randomized or Controlled Trial in title?

Introduction/aims

_____ 2. □ Background and rationale described: Yes/No

_____ 3. □ Aims given: 1° outcome(s): _____
2° outcome(s): _____

Study Population:

_____ 4. □ Setting (Inpatient or outpatient, number of sites, etc. or any of below):
Disease (condition) ____ PUD _____ Gastritis ____ Dyspepsia _____Mixed
or _____ asymptomatic carrier
Adult or pediatric, or mixed
Age range:
Country:

_____ 5. □ If recruitment/study stopped early (reason given?, na if not stopped early)

Methods

_____ 6. □ Prospective study design

_____ 7. □ Eligibility/exclusion criteria described

_____ 8. □ Sample size calculations given

_____ 9. □ Interim Analysis (yes/no or na, if not done)

_____ 10. □ Statistical methods described (yes/no)

_____ 11. □ Recruitment methods or population described, referred from hosp/clinic? (yes/no)

_____ 12. □ Subgroup analysis methods described a priori or na (if no-sub-group done)

Intervention:

_____ 13. □ Intervention well described (strain, dose, duration) (+1 if most done below)
Probiotic strain(s):
Daily dose (cfu/d):
Duration intervention period (txt time):
Duration follow-up (post-intervention):
Formulation (capsule, yogurt, milk/drink, sachet, tablet, other, not described)
Type of control (placebo, no placebo/eradication therapy only, other):

Hp eradication therapy given (double/triple/quadruple/sequential/none): Duration:

Randomization (selection bias)

_____ 14. □ Method to generate random numbers described (blocked, computer)

_____ 15. □ Balanced randomization allocation achieved (yes/no)
Probiotic group: *n* = _____ Control group: *n* =_____

Blinding (detection bias)

_____ 16. □ Blinded (single or double = +1 point) versus an open study (0 points)

_____ 17. □ Control concealment done (yes/no) [same appearance, taste, *etc*.]
Allocation concealment method described

Results: Attrition (attrition bias)

_____ 18. □ Attrition rates given by group (yes/no)

_____ 19. □ Reasons for attrition described by group (yes/no)

Outcomes (reporting bias)

_____ 20. □ Data or text comparing baseline of two groups (demographics, *etc*.)

_____ 21. □ Consort Flow-chart figure done (required post-2006)

Our primary outcome: Hp eradication

_____ 22. □ Primary-Intention to treat analysis? (+1) *vs* As-per-protocol (excludes drop-outs) (0) ?
How was primary outcome assessed?
(___13 C-urea breath test, ___histology, ___ serology, __culture, ___other)

_____23. □ Primary outcome data provided (see table below) (+1 if provided, 0 if not done)

outcome	Probiotic-arm #1	Probiotic-arm #2	Probiotic-arm #3	Probiotic-arm #4	Control	power
HP eradication (Hp negative)						
still Hp+						
totals						
P value:						

Our secondary outcome: Prevention of any adverse events

_____ 24. □ Was either AE or AAD Intention to treat analysis (+1) vs As-per-protocol (excludes drop-outs) (0) ?
How were Adverse events assessed? ____Diary ____Survey Other:_____

_____ 25. □ Outcome data provided (see table below) (+1 if provided, 0 if not done)

	Probiotic-arm #1	Probiotic-arm #2	Probiotic-arm #3	Probiotic-arm #4	Control	power
Any AE:						
No AEs noted						
Totals						
P value:						

Our Secondary outcome: Prevention of antibiotic-associated diarrhea (AAD)

_____ 26. □ AAD data given per group (+1 if provided, 0 if not done)

	Probiotic-arm #1	Probiotic-arm #2	Probiotic-arm #3	Probiotic-arm #4	Control	power
AAD						
No AAD						
Totals						
P value:						

or Description of adverse events:

Types of Adverse Events	Probiotic	Control	power

Sub-group analysis (if done)

_____ 27. □ Sub-group analysis results presented? (n/a if not done)

What were they?_____

Other bias: Discussion:

_____ 28. □ Limitations discussed

Types of limitations found: _____

_____ 29. □ Generalisability discussed (yes/no)

_____ 30. □ Compare these results to other studies (yes/no)

_____ 31. □ Trial registration number/trial registry given (for United States or European studies. post-2006)

_____ 32. □ Location where protocol can be found described (post-2006)

_____ 33. □ Source of funding given (in acknowledgements, elsewhere, or if none)

_____

Quality score (of 33 items):

Reviewer #1 _____: _____# items present (#p), _____ #items absent (#a) _____#n/a (not applicable)

Total score (#p/#p + #a) = _____

Reviewer #2 _____: _____# items present (#p), _____ #items absent (#a) _____#n/a (not applicable)

Total score (#p/#p + #a) = _____

% agreement: _____%

Figure 1 Standardized data extraction form. Scoring: For each of 33 items: +1 if numbered item is present, 0 if absent, or na (not applicable).

Outcomes and definitions

Three outcomes were assessed by this meta-analysis review: (1) eradication rates of *H. pylori*; (2) frequency of adverse events; and (3) frequency of AAD. The outcome for *H. pylori* eradication was defined by having a positive assay (pre-intervention) and a negative *H. pylori* assay done after the intervention was completed. *H. pylori* infection was diagnosed using at least one of the following assays: ^{14}C urea breath test, histology, serology, rapid urease test, stool test or culture[7]. The outcome for adverse events (AE) included any symptoms associated with eradication therapy (nausea, bloating, vomiting, diarrhea, metallic taste) were grouped as "any AE". The outcome for AAD was defined as reported diarrhea or colitis, which developed during the intervention or during the follow-up periods.

Assessment of methodological quality

Quality components for each trial were assessed for selection, detection, performance, reporting and loss to follow-up bias. Each of the included studies was evaluated using 33 items collected with the standardized data extraction form. Each item was graded as: present, absent, or not applicable (for example studies done in countries not requiring clinical trial registration, CONSORT flow-chart not present if trial was published before this became a standard, *etc.*)[28]. The overall quality score for the trial was calculated as the percent of items present divided by the total items present and absent (not applicable items were excluded from the calculation). Each of the 33 quality items were analyzed within one of six categories of potential of bias: study design bias (trial title, setting, early stoppage, background, study aims, prospective design, eligibility criteria, sample size calculation, interim analysis, statistical methods, recruitment methods, subgroup methods, probiotic well described by strain, daily dose and duration), selection bias (randomization allocation method, balanced groups resulted), detection bias (double blinded, treatments concealment), attrition bias (rates provided and reasons by each group), reporting bias (baseline group comparison, CONSORT flow-chart, intent to treat analysis done for each outcome, incidence of each outcome provided, adverse event data provided and sub-group analysis

provided, if applicable) and miscellaneous sources of bias (limitations, generalizability and comparison with other studies in discussion, trial registration, location of protocol for access and source of funding, if appropriate). Trials were classified as high quality if > 75% of the quality items were present, moderate quality if 50%-75% were present and low quality of < 50% were present. Each trial was scored for the 33 items of quality independently by at least two reviewers and a kappa statistic was applied to test for the degree of concordance.

We also employed the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system for rating overall quality of evidence for each of the outcomes by probiotic strain or type[31,32]. Recommendation for use of each probiotic strain can be assessed by the overall strength of the evidence ["strong", many randomized controlled trials show significant protection, more benefit than risk, cost-effective or "weak", only case series or reports, limited number of small trials, *etc.*]. Quality of the evidence is graded as "high quality" (further research is unlikely to change our confidence in the estimate of the effect), or "moderate quality" (further research is likely to have an important impact on our confidence and may change the estimate of the effect), or "low quality" (further research is very likely to change our confidence in the estimate and may change the direction of the estimate of the effect).

Statistical analysis

The statistical methods of this study were reviewed by Lynne McFarland from University of Washington, who holds a PhD in Epidemiology. Statistical analysis was performed using Stata software version 12 (Stata Corporation, College Station, Texas) to calculate pooled relative risks (pRR), bias estimates and number-needed-to-treat statistics. Univariate analysis results were analyzed using χ^2 test or Fisher's exact test for small cell sizes (< 5) with a significance level of $P < 0.05$. Meta-analysis was conducted for primary outcomes (*e.g.*, eradication frequency of *H. pylori* or the rate of adverse events or AAD) using models to calculate the pooled relative risk and corresponding 95%CI using the DerSimonian Laird method. Heterogeneity across trials was evaluated using Cochran Q test based on pooled relative risks by the Mantel-Haenazel method[33]. If the studies were homogenous, a fixed effects model was used; if studies were heterogeneous, a random effect model was employed. A *P*-value < 0.05 is considered statistically significant and *P*-values between 0.05 and 0.1 had a significant trend. The models used in this analysis were weighted by sample size, as study quality did not improve the fit.

If significant heterogeneity was found, subgroup analyses were conducted to determine the potential sources of heterogeneity. To explore possible explanations for heterogeneity, *a priori* subgroup analyses were conducted on study population (adult *vs* pediatric and asymptomatic versus symptomatic), daily dose [$\geqslant 1 \times 10^9$ colony-forming units (cfu) per day or $< 1 \times 10^9$ cfu/d] and study quality. A meta-regression was done without the subgroup indicator and compared to a model with the subgroup indicator included. The difference in tau^2 estimates from the two models indicates the proportion of study heterogeneity explained by the subgroup covariate (between study variance).

Publication bias

To assess for publication bias, a funnel plot, as well as a weighted regression (Egger's test) and a rank correlation test (Begg's test for small study effects) were conducted[27,34]. Funnel plots show graphically that as sample sizes of trials increase, the precision is estimating the underlying treatment effect increases, which results in the effect estimates (relative risks) from small trials scattering more widely at the bottom of the graph and narrower scattering among larger studies. In the absence of publication bias, the funnel plot resembles a symmetrical inverted funnel. Reporting bias (smaller studies showing no protective effect) often are not published, and are indicated by an asymmetrical appearance with a gap in the bottom left of a funnel plot[35,36].

RESULTS

Initial screening of data search

The literature review yielded 301 abstracts relating to probiotics and *H. pylori* that were screened for inclusion. Of those, 225 were excluded after initial screening according to our exclusion criteria (Figure 2): reviews (n = 143), pre-clinical animal models or phase two studies for pharmacokinetics, formulation or safety (n = 67), no control group (n = 6), not randomized (n = 5) or other miscellaneous reasons (n = 4). The literature search for probiotics and *H. pylori* infections found the earliest randomized, controlled efficacy trial was published in 2000. Literature from 1994-1999 only included early investigative studies (mechanism of action, dose-ranging and safety studies) and no clinical trials were found published before 1994.

Secondary screening of full articles

Of the 76 full articles or meeting abstracts retrieved, an additional 35 were excluded: just one RCT found, *i.e.*, no confirmatory RCTs for probiotic strain found (n = 18), no *H. pylori* eradication therapy given with probiotic (n = 11), undefined probiotic product with no species and strain identification (n = 3), no *H. pylori* assays done (n = 1) and two RCTs assessed the burden of *H. pylori* reduced by probiotics but did not document eradication rates nor the frequency of adverse events. Of these trials assessing probiotics

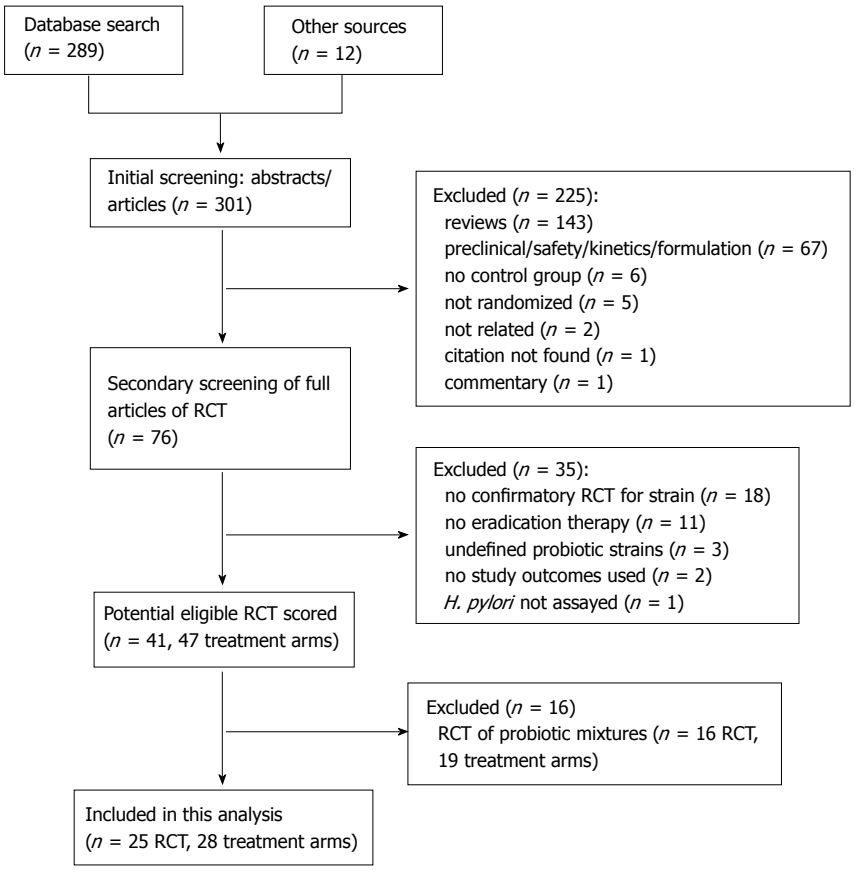

Figure 2 Flow chart of included and excluded trials for Helicobacter pylori eradication/adverse events. RCT: Randomized controlled trials.

and *H. pylori* eradication and/or side effects, 25 (61%) were testing a single strain of probiotic and were included in this analysis and 16 (39%) used multiple strains of probiotics and will be addressed elsewhere. Data extraction was performed independently by co-authors on the remaining 25 RCTs. Examples of RCTs included in prior published meta-analyses, but excluded in our analysis, are shown in Table 2. Reasons for excluding RCTs included: other types of outcomes were assessed[37-39], no concurrent *H. pylori* eradication therapy given[40-46], only one RCT for a specific strain was found[47-52].

Included trials

Of the 25 randomized controlled trials included[53-77], several had multiple treatment arms[53,57,65], resulting in 28 treatment arms, totaling 3769 participants. The sample sizes of the trials ranged from 12 to 991, with a mean number per trial of 68 ± 84 in probiotic arms and 66 ± 83 in control arms. Three articles were translated from their original languages into English: Chinese[62,63] or Spanish[69]. Only two articles were from published meeting abstracts[72,77] with no subsequent full article publications found, the remaining were peer-reviewed full articles.

Patient population

The characteristics of the enrolled study populations by trial arm are presented in Table 3. Of the 28 treatment arms, most enrolled adult participants (*n* = 24, 86%) and four (14%) enrolled children and all trials included both genders. Race or ethnicity was not reported in most clinical trials. The trials were carried out in a wide array of countries: Italy (40%), Turkey (12%), China (12%), Japan (8%), South Korea (8%) and one trial each (4%) for the following: Greece, Iran, Poland, Romania and Venezuela. All treatment arms enrolled *H. pylori* positive participants who were either symptomatic (*n* = 21, 75%), or asymptomatic carriers (*n* = 5, 18%), or had a mixed population (*n* = 1) but one RCT did not report symptom status at enrollment.

Study design

Randomization: All 28 RCT were randomized, but only 12 (43%) provided the method used to randomize patients (*e.g.*, computer random number generator, random block design).

Degree of blinding: Of the 28 treatment arms, only seven arms (25%) were double-blinded (used placebos that were of identical appearance as the probiotic formulation)[53,55,67-69,73], four arms (14%) were single blinded (either participants were unaware of the other treatment arm[61] or outcome assessor was blinded)[56,57]. Most, 17 (61%) of the treatment arms were open trials (no placebos and participants were aware that there was another treatment arm),

Table 2 Excluded randomized controlled trials

Probiotic strain	Reason for exclusion	Ref.
L. gasseri OLL2716	Study quality poor for treatment arm	Boonyaritichaikij *et al*[37]
L. rhamnosus GG	No *H. pylori* assay done	Gawrońska *et al*[38]
L. reuteri ATCC 55730	Outcome was *H. pylori* burden	Francavilla *et al*[39]
S. boulardii I-745 or *L. acidophilus* Lb	No eradication therapy given with probiotic	Gottleland *et al*[40]
Bifido. bifidum YIT4007	No eradication therapy given with probiotic	Miki *et al*[41]
L. casei Shirota	No eradication therapy given with probiotic	Cats *et al*[42]
L. gasseri OLL2716	No eradication therapy given with probiotic	Takagi *et al*[43]
L. johnsonii Lj1	No eradication therapy given with probiotic	Pantoflickova *et al*[44]
L. johnsonii Lj1	No eradication therapy given with probiotic	Gottleland *et al*[45]
L. reuteri ATCC 55730	No eradication therapy given with probiotic	Saggioro *et al*[46]
Bacillus clausii nr	< 2 RCT with eradication therapy	Nista *et al*[47]
Bifido. animalis DN173010	< 2 RCT with eradication therapy	Yaşar *et al*[48]
Bifido. infantis 2036	< 2 RCT with eradication therapy	Dajani *et al*[49]
L. johnsonii Lc-1	< 2 RCT with eradication therapy	Felley *et al*[50]
L. casei DN 114001	< 2 RCT with eradication therapy	Sýkora *et al*[51]
L. casei Shirota	< 2 RCT with eradication therapy	Sahagún-Flores *et al*[52]

nr: Strain not reported; RCT: Randomized controlled trials.

Table 3 Characteristics of enrolled populations in patients receiving eradication therapy by 28 treatment arms

Probiotic strain	Country	Population	Symptoms	Blinding	Eradication therapy	Duration eradication (d)	Ref.
S. boulardii I-745	Italy	Adults	Asymptomatic	Placebo	CTR	7	Cremonini *et al*[53]
S. boulardii I-745	Turkey	Adults	Symptomatic	None	ACO	14	Duman *et al*[54]
S. boulardii I-745	Turkey	Adults	Symptomatic	Placebo	ACL	14	Cindoruk *et al*[55]
S. boulardii I-745	Romania	Pediatric	Symptomatic	Single	AC + O/E	7-21	Hurduc *et al*[56]
S. boulardii I-745	South Korea	Adults	Symptomatic	Single	ACO	7	Song *et al*[57]
S. boulardii I-745 + MPA	South Korea	Adults	Symptomatic	Single	ACO	7	Song *et al*[57]
S. boulardii I-745	Turkey	Adults	Symptomatic	None	ACL	14	Ozdil *et al*[58]
S. boulardii I-745	China	Adults	Symptomatic	None	ACO	14	Chu *et al*[59]
S. boulardii I-745	Iran	Adults	Symptomatic	None	ACO	14	Zojaji *et al*[60]
S. boulardii I-745	Greece	Adults	Symptomatic	Single	ACO	14	Kyriakos *et al*[61]
S. boulardii I-745	China	Pediatric	Symptomatic	None	ACO	14	Zhao *et al*[62]
Clost. butyricum 588	China	Adults	Symptomatic	None	AFO	7	Guo *et al*[63]
Clost. butyricum 588	Japan	Adults	Symptomatic	None	ACL	7	Shimbo *et al*[64]
Clost. butyricum 588 (low dose)	Japan	Adults	Symptomatic	None	ACL	7	Imase *et al*[65]
Clost. butyricum 588 (high dose)	Japan	Adults	Symptomatic	None	ACL	7	Imase *et al*[65]
L. rhamnosus GG	Italy	Adults	Asymptomatic	None	CPT	7	Armuzzi *et al*[66]
L. rhamnosus GG	Italy	Adults	Asymptomatic	Placebo	CRT	7	Armuzzi *et al*[67]
L. rhamnosus GG	Italy	Adults	Asymptomatic	Placebo	CRT	7	Cremonini *et al*[53]
L. rhamnosus GG	Poland	Pediatric	Asymptomatic	Placebo	ACO	7	Szajewska *et al*[68]
L. rhamnosus GG	Venezuela	Adults	Symptomatic	Placebo	ACO	7	Padilla Ruiz *et al*[69]
L. acidophilus Lb	Italy	Adults	Symptomatic	None	ACR	7	Canducci *et al*[70]
L. acidophilus Lb	Italy	Adults	Symptomatic	None	AO	7-30	De Francesco *et al*[71]
L. acidophilus nr	South Korea	Adults	Mixed	None	ACO	7	Yeom *et al*[72]
L. reuteri 55730	Italy	Pediatric	Symptomatic	Placebo	AO, COT	15	Lionetti *et al*[73]
L. reuteri 55730	Italy	Adults	Symptomatic	None	ACT	7	Scaccianoce *et al*[74]
L. reuteri 55730	Italy	Adults	Symptomatic	None	AELe	7	Ojetti *et al*[75]
L. casei DG	Italy	Adults	Symptomatic	None	ART (E/P)	10	Tursi *et al*[76]
L. casei DG	Italy	Adults	nr	None	ACE	7	Giovannone *et al*[77]

This strain is now designated: Saccharomyces boulardii CNCM I-745. Clostridium butyricum 588 (MIYAIRI). Placebo indicates double-blinded design, single indicates either just patient or outcome assessor was blinded and none indicates an open study. A: Amoxicillin; C: Clarithromycin; E: Esomeprazole; F: Furazolidone; L: Lansoprazole; Le: Levofloxacin; MPA: Mucoprotective agent; nr: Not reported in paper/abstract; O: Omeprazole; P: Pantoprazole; R: Randazole; T: Tindazole.

as shown in Table 3.

***H. pylori* eradication therapy**: All trials were required to use an *H. pylori* eradication therapy, which included at least one antibiotic and one PPI for both the probiotic and control group (Table 3). Of the 28 treatment arms, only 1 (4%) used double therapy (amoxicillin and omeprazole)[71]. Most used triple therapy (n = 25, 89%), which most commonly included two antibiotics (amoxicillin and clarithyromycin) combined with a PPI

(omeprazole). Less commonly used were quadruple therapy (n = 1 arm, 4%) or sequential therapy (n = 1 arm, 4%). Overall, the duration of eradication therapy ranged from one week (61% of treatment arms), to 10 d (3%), to two weeks (29%) or varied from 1-4 wk (7%).

Attrition: Attrition ranged from 0%-27% in the 28 treatment arms, usually due to drop-outs due to adverse events or loss to follow-up. Fourteen treatment arms (50%) reported no attrition, 10 (36%) had attrition frequencies from 1%-10% and only three (11%) reported higher attrition (11%-27%), while one trial did not document attrition rates. Of the 28 treatment arms, 24 (86%) used ITT analysis and four (14%) used APP analysis. However, only three of the trials reported how the ITT analysis incorporated the missing data (treated all missing outcomes as failures)[61,63,70].

Intervention

Details of the intervention for the 25 RCT (28 treatment arms) are given in Tables 4 and 5.

Type of probiotic strain(s): In the 28 treatment arms, six different single strain probiotic types were assessed (Tables 3-5) by at least two RCTs that met our eligibility criteria. The most commonly tested strain is *S. boulardii* CNCM I-745, with 11 (39% of RCT arms). *Lactobacillus rhamnosus* (*L. rhamnosus*) GG was tested in five arms (18%), *Clostridium butyricum* 588 was tested in four arms (14%), *L. reuteri* ATCC 55730 and *L. acidophilus* Lb were each tested in two (7%) treatment arms and *L. casei* DG was tested in two treatment arms (7%), one strain of *L. acidophilus* could not be determined.

Newer strain designations for several probiotics and the retrospective review of older studies may have used different strain designations, but were, in fact, the same strain. The most recent strain designations are used in this study. The most current strain designation for *S. boulardii* is CNCM I-745, the registration number at the Pasteur Institute[78], but older studies also refer to this strain as *S. boulardii* lyo, or *S. boulardii*, with no strain designation. *Clostridium butyricum* 588 was also known as *C. butyricum* MIYAIRI. The strain of *L. acidophilus* in one study was referred to only by the brand name (Antibio, China) in the meeting abstract and correspondence with authors and manufacturers were unproductive, but this strain was included in the analysis to illustrate the importance of providing strain designations[72].

Probiotic dose: The daily dose of probiotics varied widely from 1×10^6 to 2×10^{10} colony-forming units (cfu) per day. The *a priori* subgroup analyses on dose compared high dose probiotic ($\geqslant 1 \times 10^9$ cfu/d) versus low dose ($< 1 \times 10^9$ cfu/d). Nineteen (68%) of the treatment arms used the higher daily dose of probiotics and nine (32%) used lower doses (Table 4). The daily dose was reported in all trials, but in some cases the dose was reported as mg/d not cfu/d and required conversion.

Formulation used: Most of the 28 treatment arms used a capsule formulation (12 arms, 43%), while six (21%) used sachets, six (21%) used tablets, two (7%) used liquid and the formulation was not reported in two (7%) of the studies.

Probiotic duration: The probiotics were typically administered as an adjunct for the same duration as the standard eradication therapy, but some RCT continued the probiotic/control intervention for an additional week. The most frequent duration of probiotic was for 2 wk (16 arms, 57%), while five (18%) gave probiotics for only one week and four (14%) gave probiotics for three weeks. Two treatment arms gave probiotics for 10 d (7%) and one (4%) gave for 20 d. All trials reported duration of probiotic given (Table 4).

Length of follow-up: In most trials, participants were followed and tested for *H. pylori* presence 4-8 wk after the intervention treatments were discontinued. Of the 28 treatment arms, 21 (75%) had 1-7 wk of follow-up and four (14%) had longer follow-up times, while three (11%) did not report any follow-up times (Table 4).

Efficacy of adjunct probiotics for H. pylori eradication

Of the 28 treatment arms, 26 (93%) reported *H. pylori* eradication rates in their paper. A low amount of heterogeneity was found when all strains were pooled together (I^2 =25%, P = 0.12), thus a fixed effects model was used for this outcome. The overall pooled RR indicated that probiotics, in general, were effective for *H. pylori* eradication (pRR = 1.10, 95%CI: 1.06-1.14) with a number-needed-to-treat (NNT) of 14. However, as recommended by the literature[24,79], the efficacy should be assessed separately by probiotic strain, as shown by the forest plot (Figure 3). This figure shows that only *S. boulardii* I-745 (n = 10 treatment arms, pRR = 1.11, 95%CI: 1.07-1.16) was significantly effective as an adjunct for *H. pylori* eradication. None of the pooled RR from the other five strains (*C. butyricum* 588, *L. rhamnosus GG*, *L. acidophilus* Lb, *L. reuteri* 55730 or *L. casei* DG significantly improved *H. pylori* eradiation rates with standard therapy. Deletion of the trial with the unknown strain of *L. acidophilus* did not significantly affect the pooled RR estimates.

Sub-group analysis: Results from the meta-regression analysis for the adjunctive use of probiotics for *H. pylori* eradication did not find significant differences in associations between the study population (adult

Table 4 Description of the interventions and *Helicobacter pylori* eradication rates *n* (%)

Probiotic strain	Daily dose (cfu/d)	Form	Duration treatment (wk)	Follow-up post-treatment (wk)	*H. pylori* eradication probiotic	*H. pylori* eradication in controls	Ref.
S. boulardii I-745	1×10^{10}	Sachet	2	5-7	17 (81)	16 (80)	Cremonini *et al*[53]
S. boulardii I-745	1×10^{10}	Capsule	2	4	nr	nr	Duman *et al*[54]
S. *boulardii* I-745	2×10^{10}	Sachet	2	6	44 (71)	37 (60)	Cindoruk *et al*[55]
S. boulardii I-745	1×10^{10}	Capsule	4	4-6	45 (93.3)	34 (80.9)	Hurduc *et al*[56]
S. boulardii I-745	2×10^{10}	Capsule	4	4	264 (80)[a]	237 (71.6)	Song *et al*[57]
S. boulardii I-745 + MPA	2×10^{10}	Capsule	4	4	271 (82.1)[b]	237 (71.6)	Song *et al*[57]
S. boulardii I-745	5×10^{9}	Capsule	2	5	71 (72)	82 (86)[a]	Ozdil *et al*[58]
S. boulardii I-745	5×10^{9}	Sachet	2	52	42 (84)[a]	32 (64)	Chu *et al*[59]
S. boulardii I-745	1×10^{10}	Capsule	2	8	70 (87.5)	65 (81)	Zojaji *et al*[60]
S. boulardii I-745	6×10^{6}	Capsule	2	6	30 (83.4)[a]	20 (58.8)	Kyriakos *et al*[61]
S. boulardii I-745	1×10^{10}	Capsule	2	4	102 (85)[c]	91 (75.8)	Zhao *et al*[62]
Clost. butyricum 588	1×10^{7}	Tablet	1	4	44 (94)	44 (88)	Guo *et al*[63]
Clost. butyricum 588	3×10^{7}	Tablet	2	6	17 (94)	13 (76)	Shimbo *et al*[64]
Clost. butyricum 588 (low dose)	6×10^{7}	Tablet	1	0	7 (100)	6 (87)	Imase *et al*[65]
Clost. butyricum 588 (high dose)	1.2×10^{8}	Tablet	1	0	4 (80)	6 (87)	Imase *et al*[65]
L. rhamnosus GG	1.2×10^{10}	Sachet	2	6	48 (80)	46 (76.6)	Armuzzi *et al*[66]
L. rhamnosus GG	1.2×10^{10}	Sachet	2	6	25 (83)	24 (80)	Armuzzi *et al*[67]
L. rhamnosus GG	1.2×10^{10}	Sachet	2	5-7	16 (76)	16 (80)	Cremonini *et al*[53]
L. rhamnosus GG	2×10^{9}	Capsule	1	6	23 (69)	22 (68)	Szajewska *et al*[68]
L. rhamnosus GG	1.2×10^{10}	Liquid	2	0	nr	nr	Padilla Ruiz *et al*[69]
L. acidophilus Lb	1.5×10^{10}	Capsule	1.4	6	52 (87)[a]	42 (70)	Canducci *et al*[70]
L. acidophilus Lb	2×10^{10}	Capsule	2	4-6	30 (64)	26 (70)	De Francesco *et al*[71]
L. acidophilus nr	2×10^{8}	nr	2	4-8	19 (83)	21 (95.5)	Yeom *et al*[72]
L. reuteri 55730	1×10^{8}	Tablet	2.9	8	17 (85)	16 (80)	Lionetti *et al*[73]
L. reuteri 55730	2×10^{8}	Tablet	1	4-6	9 (53)	10 (62)	Scaccianoce *et al*[74]
L. reuteri 55730	3×10^{8}	Liquid	2	6	36 (80)[a]	27 (60)	Ojetti *et al*[75]
L. casei DG	1.6×10^{10}	Capsule	1.4	4	33 (94.3)	30 (85.7)	Tursi *et al*[76]
L. casei DG	2×10^{10}	nr	4	6	22 (73)	21 (70)	Giovannone *et al*[77]

[a]$P < 0.05$, [b]$P < 0.01$, [c]Trend, $0.05 \leqslant P < 1.0$. This strain is now designated: Saccharomyces boulardii CNCM I-745, nr: Not reported; *S. boulardii*: *Saccharomcyes boulardii; L. rhamnosus: Lactobacillus rhamnosus.*

versus pediatric, $P = 0.76$), baseline disease state (asymptomatic carriage versus symptoms, $P = 0.17$), daily dose of probiotic (above or below 10^9 cfu/d, $P = 0.26$), or study quality ($P = 0.11$). Only probiotic strain group showed significance, confirming the validity of analyzing efficacy by strain type. Sub-group analysis for duration probiotic given and by type of *H. pylori* eradication therapy was not possible, as most trials used similar durations and types of eradication therapy.

Efficacy of adjunct probiotics for prevention of any adverse events

Of the 28 treatment arms, 18 (64%) planned *a priori* to document any adverse events that might occur during the intervention and follow-up period (if done), while 10 (36%) did not document total adverse events during their trials (Table 5). Overall, the pooled RR showed a protective effect (pRR = 0.54, 95%CI: 0.42-0.70, NNT = 8), and as significant heterogeneity was found (I^2 = 56%, $P = 0.003$), random effects models were used for this outcome. The forest plot (Figure 4) shows that only *S. boulardii* I-745 ($n = 7$ treatment arms, pRR = 0.42, 95%CI: 0.28-0.62) significantly reduced the incidence of adverse events associated with standard *H. pylori* eradication therapies. *L. acidophilus* Lb and *L. rhamnosus* GG had no significant protective effect for adverse events and the other three strains of probiotics only had a single treatment arm evaluating adverse events.

Efficacy of adjunct probiotics for the prevention of antibiotic associated diarrhea

Of the 28 treatment arms, 20 (71%) planned *a priori* to document AAD during the intervention and follow-up period (if done), while eight (29%) did not document AAD outcomes (Table 5). Overall, the pooled RR showed a protective effect (pRR = 0.43, 95%CI: 0.35-0.53, NNT = 10), and as significant heterogeneity was not found ($I^2 = 0$, $P = 0.88$), fixed effects models were used to summarize AAD trials. The forest plot (Figure 5) shows that only *S. boulardii* I-745 (n = 9 treatment arms, pRR = 0.47, 95%CI: 0.37-0.60) and *L. rhamnosus* GG ($n = 5$ treatment arms, pRR = 0.29, 95%CI: 0.17-0.48) significantly reduced the incidence of AAD associated with *H. pylori* eradication therapy. The pooled RR from *C. butyricum* 588 and *L. reuteri* 55730 did not find a significant protective effect on AAD. Two strains (*L. acidophilus* Lb and *L. casei* DG) could not be assessed with pooled RRs due to insufficient trials with AAD outcome data.

Publication bias

A funnel plot analysis (Figure 6) provides no compelling

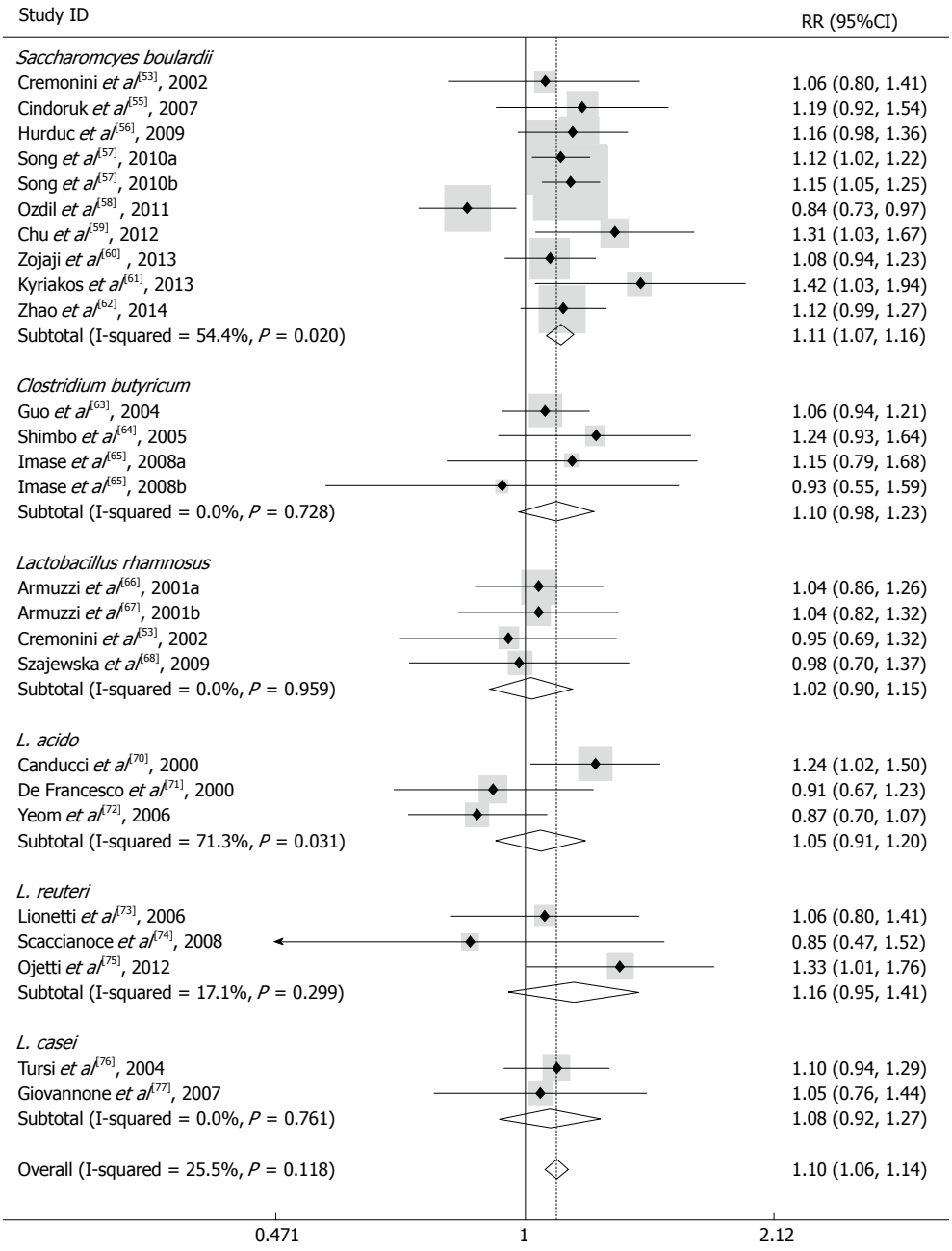

Figure 3 Forest plot of *Helicobacter pylori* eradication by probiotic strain.

indication of publication bias for trials evaluating *H. pylori* eradication outcomes, showing general symmetry of the funnel for the relationship between risk ratio and standard error. The funnel plot shows a lack of published small sized trials with an improved eradication rate. However, Egger's regression test for small study effects (P = 0.71) and Begg's rank test (P = 0.37) fail to suggest significant publication bias. No significant publication bias was found for the RCT assessing the prevention of all adverse reactions (Egger's regression P = 0.42 and Begg's rank P = 0.74). Potential publication bias may be present in RCTs assessing AAD (Egger's regression P = 0.003 and Begg's rank P = 0.025), as there were few outliers noted for small study sizes (Figure 7).

Quality of studies

Of the 25 RCTs, 3 (12%) were rated as high quality studies, 18 (72%) moderate quality and 4 (16%) were low quality trials. The concordance from the reviewers was acceptable (kappa = 0.62, P < 0.001) and any disagreements typically involved only 1-2 of the 33 items in the data extraction form. All disagreements were resolved. As shown in Figure 8, most trials had high quality study design (60%), but only 16% included sample size calculations, 76% failed to indicate "randomized controlled trial" in the title and only 48% described how participants were recruited. There were a low number of trials with selection bias, as all were randomized, but only 40% described the method of randomization used. There was a high

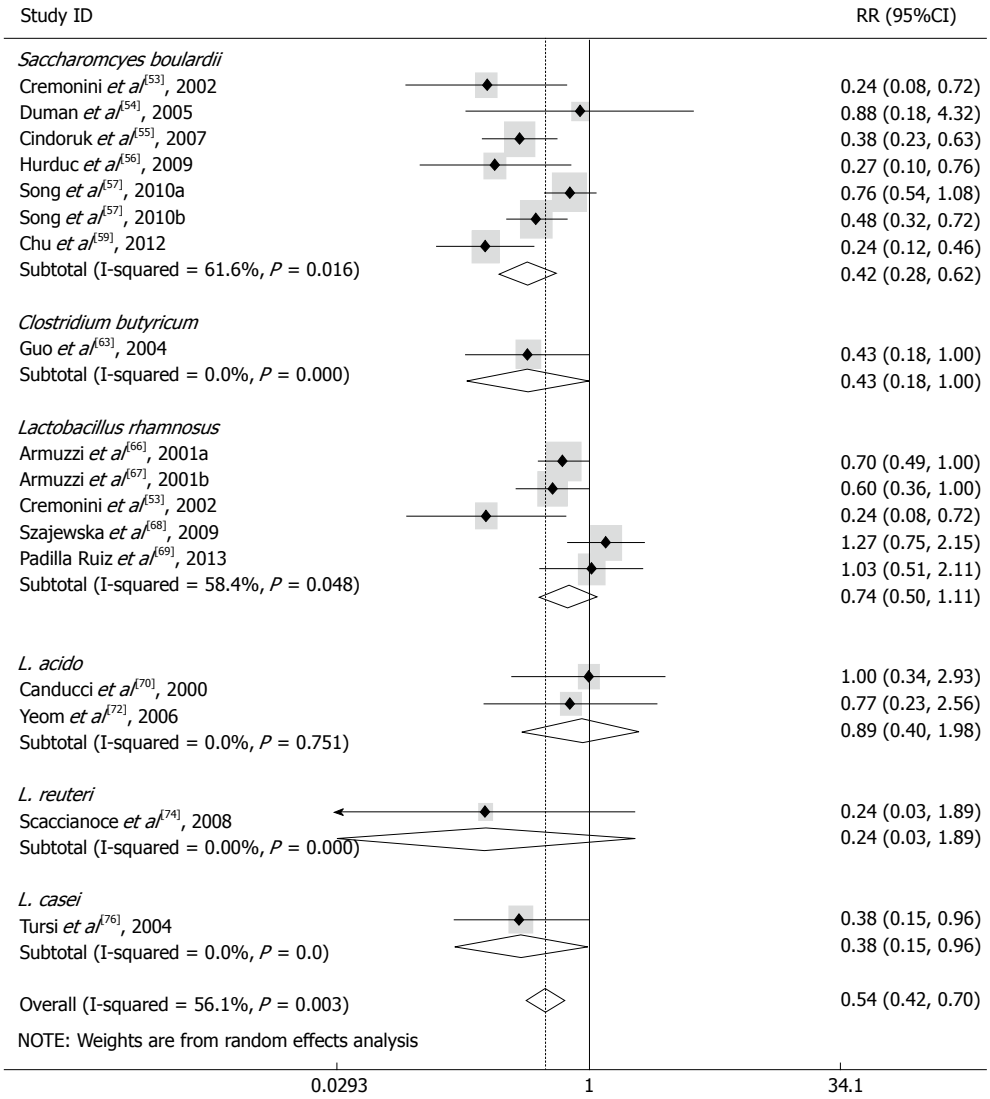

Figure 4 Forest plot of any adverse events by probiotic strain.

degree of detection bias due to the frequent used of open study designs (only 40% were double-blinded) and only 24% described the method of treatment concealment. Most (80%) of the trials reported their attrition rates, but only 65% provided the reasons for attrition by treatment groups. Reporting bias of the outcomes was generally high-moderate quality, but only 44% provided a consort figure describing study flow and only 56% provided a comparison of the two treatment groups at baseline. Other sources of bias were typically of poor quality due to the lack of trial registration or funding source descriptions. In the discussion section of the papers, although 84% compared their results to other studies, only 36% discussed limitations and few (8%) discussed generalizability of their results.

GRADE criteria

For the *H. pylori* eradication, we recommend the following adjunct probiotic strains: *S. boulardii* CNCM I-745 (high quality and strong strength). For the prevention of adverse events associated with standard *H. pylori* eradication therapy, we recommend *S. boulardii* CNCM I-745 (high quality and strong strength). For the prevention of antibiotic-associated diarrhea associated with standard *H. pylori* eradication therapy, we recommend the following adjunct probiotic strains: *S. boulardii* CNCM I-745 (high quality and strong strength) and *L. rhamnosus GG* (strong quality and strong strength). All other strains require additional multiple randomized, controlled trials before a recommendation can be provided.

DISCUSSION

Our meta-analyses found only one probiotic strain significantly improved *H. pylori* eradication rates: *S. boulardii* CNCM I-745 (pRR = 1.11, 95%CI: 1.07-1.15). Only one probiotic strain (*S. boulardii* CNCM I-745) significantly prevented any adverse events (pRR = 0.42, 95%CI: 0.28-0.62). Two probiotic strains significantly reduced antibiotic-associated diarrhea, *S. boulardii* CNCM I-745 and *L. rhamnosus* GG (pRR = 0.47, 95%CI: 0.37-0.60 and pRR = 0.29, 95%CI:

Table 5 Prevention of adverse events associated with *Helicobacter pylori* eradication therapy in 28 treatment arms with adjunct probiotics *n* (%)

Probiotic strain	Any adverse events in probiotic	Any adverse events in controls	Antibiotic associated diarrhea in probiotic	Antibiotic associated diarrhea in controls	Ref.
S. boulardii I-745	3 (14)[b]	12 (60)	1 (5)[a]	6 (30)	Cremonini *et al*[53]
S. boulardii I-745	3 (1.5)	3 (1.7)	14 (6.9)[b]	28 (15.6)	Duman *et al*[54]
S. boulardii I-745	14 (23)[b]	37 (60)	9 (14.5)[a]	19 (30.6)	Cindoruk *et al*[55]
S. boulardii I-745	4 (8)[a]	13 (31)	nr	nr	Hurduc *et al*[56]
S. boulardii I-745	48 (14)	63 (19)	9 (3.3)[a]	20 (6)	Song *et al*[57]
S. boulardii I-745 + MPA	30 (9)[b]	63 (19)	11 (3)	20 (6)	Song *et al*[57]
S. boulardii I-745	nr	nr	nr	nr	Ozdil *et al*[58]
S. boulardii I-745	8 (16)[b]	34 (68)	3 (6)	8 (16)	Chu *et al*[59]
S. boulardii I-745	nr	nr	10 (12.5)[a]	21 (26)	Zojaji *et al*[60]
S. boulardii I-745	nr	nr	1 (2.8)[a]	7 (20.6)	Kyriakos *et al*[61]
S. boulardii I-745	nr	nr	27 (22.5)[b]	47 (39.1)	Zhao *et al*[62]
Clost. butyricum 588	6 (12.8)[a]	15 (30)	nr	nr	Guo *et al*[63]
Clost. butyricum 588	nr	nr	1 (6)	2 (11.8)	Shimbo *et al*[64]
Clost. butyricum 588 (low dose)	nr	nr	1 (14)	3 (43)	Imase *et al*[65]
Clost. butyricum 588 (high dose)	nr	nr	0 (0)	3 (43)	Imase *et al*[65]
L. rhamnosus GG	26 (43)[a]	37 (62)	8 (13.2)[b]	29 (48.2)	Armuzzi *et al*[66]
L. rhamnosus GG	12 (40)[a]	20 (66.6)	1 (3.3)[b]	8 (26.6)	Armuzzi *et al*[67]
L. rhamnosus GG	3 (15)[b]	12 (60)	1 (5)	6 (30)	Cremonini *et al*[53]
L. rhamnosus GG	18 (51)	13 (41)	2 (6)	6 (20)	Szajewska *et al*[68]
L. rhamnosus GG	10 (34)	10 (33)	4 (13.8)	6 (20)	Padilla Ruiz *et al*[69]
L. acidophilus Lb	6 (10)	6 (10)	nr	nr	Canducci *et al*[70]
L. acidophilus Lb	nr	nr	nr	nr	De Francesco *et al*[71]
L. acidophilus nr	4 (15)	5 (19)	nr	nr	Yeom *et al*[72]
L. reuteri 55730	0 (0)	0 (0)	nr	nr	Lionetti *et al*[73]
L. reuteri 55730	1 (5.9)[c]	4 (26.7)	0 (0)[a]	2 (13)	Scaccianoce *et al*[74]
L. reuteri 55730	nr	nr	10 (22)[b]	26 (58)	Ojetti *et al*[75]
L. casei DG	5 (14.3)[a]	13 (37)	0 (0)	3 (8.6)	Tursi *et al*[76]
L. casei DG	nr	nr	nr	nr	Giovannone *et al*[77]

[a]$P < 0.05$, [b]$P < 0.01$, [c]Trend, $0.05 \leqslant P < 1.0$. This strain is now designated: Saccharomyces boulardii CNCM I-745. nr: Not reported in paper/abstract. Numbers in table given as frequency and percent (%). *S. boulardii*: *Saccharomcyes boulardii; L. rhamnosus: Lactobacillus rhamnosus.*

0.17-0.48, respectively). The most promising probiotics strains for *H. pylori* infections also have documented mechanisms of action directed against *H. pylori*. *S. boulardii* produces a neuraminidase that attacks sialic acid, an attachment receptor for *H. pylori*[18] and also induces a morphologic change from the spiral form to a coccoid form of *H. pylori*[80]. There is no direct evidence linking *L. rhamnosus* GG to specific anti-*H. pylori* actions. However, both *S. boulardii* CNCM I-745 and *L rhamnosus* GG have been shown to prevent AAD given for other infections[15,79,81-83].

Our findings are similar to other meta-analyses of probiotics for *H. pylori* infections, which differ by including fewer numbers of trials or did not examine all three outcomes (eradication, adverse reactions and AAD). Szajewska *et al*[84] pooled five randomized trials with *S boulardii* and found significantly better *H. pylori* eradication (pRR = 1.13, 95%CI: 1.05-1.21) and significantly less AAD (pRR = 0.47, 95%CI: 0.32-0.69). Our meta-analysis confirms the robustness of this efficacy from 10 RCTs showing a mild (9%) increase in mean *H. pylori* eradication rates from 73% in control arms to 82% in *S. boulardii* arms, and a reduced rate of AAD in *S. boulardii* arms compared to control arms (8.5% and 21%, respectively). We could not find any other meta-analyses that limited their review to one probiotic strain for *H. pylori* infections.

Tactics for limiting heterogeneity due to the differences of strain-specific probiotic efficacies can be done at the beginning (inclusion criteria only allowing one strain to be included) or post-literature harvesting (by performing sub-group analysis by strain type). Tong *et al*[85] reviewed 14 randomized trials from various probiotic strains and did a sub-group analysis by the type of probiotic and reported only one strain, *L. rhamnosus* GG, showed better *H. pylori* eradication rates odds ratio (OR) from four trials (pOR = 2.09, 95%CI: 1.28-3.4), although one of those trials was actually *L. casei*, not *L. rhamnosus*[85]. Zou *et al*[86] pooled eight trials for *H. pylori* eradication, but incorrectly combined different strains in their subgroup analyses. When Zou *et al*[86] presented data for adverse event rates, they reported five RCT identified as "*L. casei*", however the data presented was actually for eradication rates and three of the five studies used *L. rhamnosus* GG, while the two other studies used different *L. casei* strains (DN11400 and DG). One of the two pooled studies identified as "*L. acidophilus*" used a mixture of two different Lactobacilli strains[86]. Some meta-analyses did not separate out probiotic strains using sub-group analysis and only presented summary risk estimates combining many different probiotic

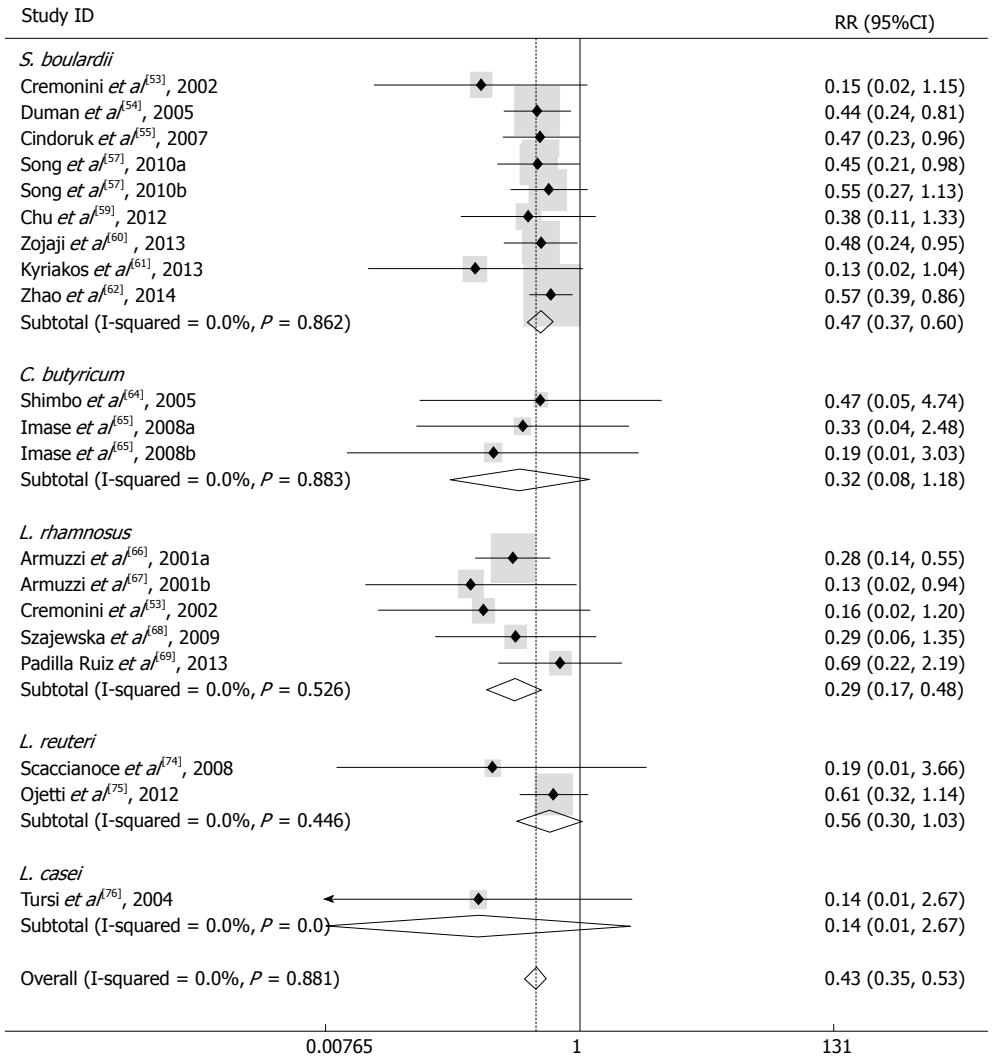

Figure 5 Forest plot of prevention of antibiotic-associated diarrhea by probiotic strain.

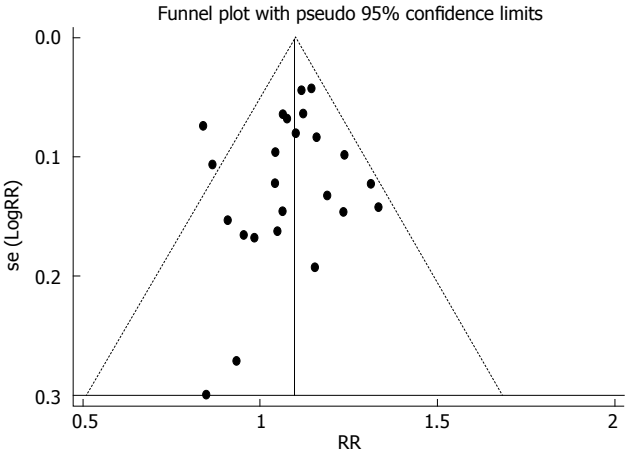

Figure 6 Funnel plot for publication bias assessment from for *Helicobacter pylori* eradication and probiotics.

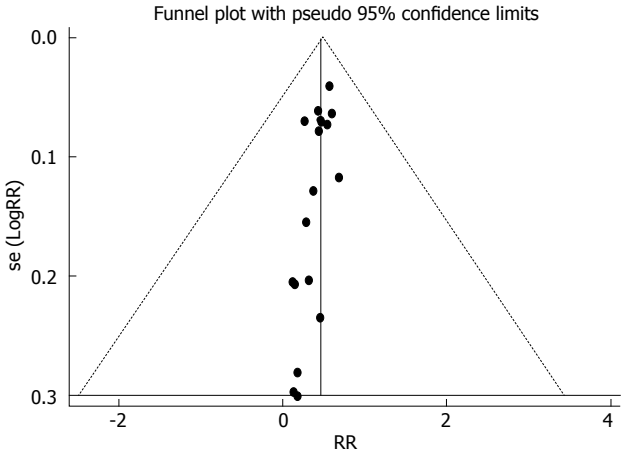

Figure 7 Funnel plot for publication bias assessment from for prevention of antibiotic associated diarrhea and probiotics.

strains[87-89]. Sachdeva *et al*[90] did not find an effect by probiotic strain in their meta-regression analysis. Wang *et al*[91] pooled 10 RCT using different mixtures containing Lactobacilli and/or Bifidobacterium and did a sub-group analysis on race, quality, symptoms, age and types of eradication therapy, but failed to analyze the strains of probiotics separately.

Other reviews and meta-analysis have also analyzed

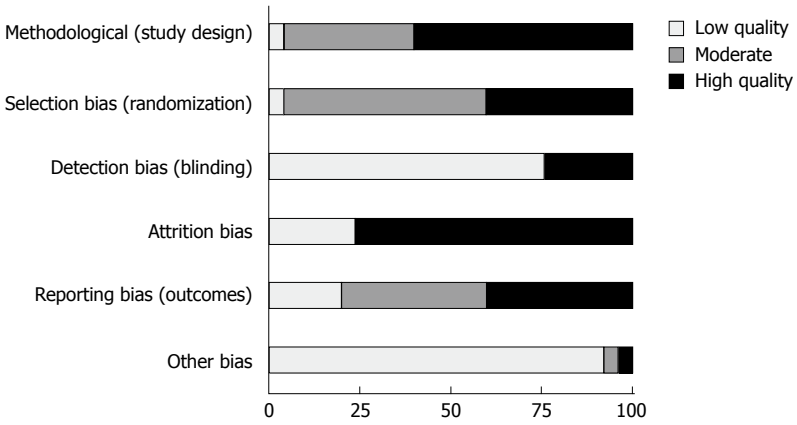

Figure 8 Frequency of study quality based on six different types of potential bias. High quality, low bias (76%-100% quality items within category present), moderate quality and moderate bias (51%-75% items present), low quality, high bias (0%-50% items present).

the effect of probiotics for the prevention of adverse events and AAD related to *H. pylori* eradication therapy, but typically have pooled different strains together into one group[85,87,89,91]. Zou *et al*[86] reported no significant effect of Lactobacilli probiotics on adverse events, but pooled together studies using *L. rhamnosus* GG (3 studies), *L. acidophilus*, *L. casei*, and *L. reuteri* (one study each) into the same group. As our meta-analysis shows a distinct strain specificity to both the efficacy of eradicating *H. pylori* and the prevention of adverse events (including AAD), future studies need to be aware that pooling similar probiotics by species is no longer appropriate and their outcomes need to analyzed by the same type of probiotic strain.

The quality of clinical trials in our analysis varied from a score of 0.32-0.89, which was not surprising as some of the trials were done before standardized randomized controlled trial guidelines were widely published and two trials with low quality scores were from meeting abstracts that never resulted in full article publications. The advantage of scoring trials on quality is the results allow an assessment of recommendations to improve future studies. Future trials would benefit from better study designs (use of placebos, study size calculations), more complete descriptions of their outcomes and discussion of limitations and generalizability.

A question that arises from discussions on how best to treat patients with *H. pylori* is whether probiotics alone are sufficient to treat these infections, or is adjunctive therapy with the standard antibiotic and PPI therapy more effective. The study by Gotteland *et al*[40] tested *S. boulardii* alone or heat-killed *L. acidophilus* Lb alone versus triple therapy *H. pylori* eradication therapies and found *S. boulardii* alone or *L. acidophilus* alone was significantly poorer (12% and 6% eradication, respectively) than triple therapy used alone (66%, $P < 0.05$), thus strengthening the position that probiotics are most effective when combined with antibiotic-PPI eradication therapy. Most other studies testing probiotics alone (without the standard eradication therapies) have failed to show a significant effect of the probiotic[41,42,44], while a few found significant improvement of eradication rates using just a probiotic[45,46], although one study treated patients with either only a PPI (omeprazole) or *L. reuteri*/PPI and did not use any antibiotics in the control group[46].

The results of the Maastricht IV/Florence Consensus, which involved 44 experts on *H. pylori,* reported the decreasing eradication rates of the triple therapy (only 70%) may be due to the development of resistance to clarithromycin and poor compliance due to adverse events associated with triple therapies[7]. This group found better eradication rates using either sequential treatments [5 d of PPI and amoxicillin followed by 5 d of PPI, clarithromycin and metronidazole (or tinidazole)] or quadruple therapy (PPI with two antibiotics and bismuth). This group also recommended extending the duration of therapy from 7 d to 10-14 d. While eradication rates may improve with these regimes, the incidence of adverse events remains high. At the time of the meeting (2010), they did not recommend the use of probiotics, citing the poor quality of the studies due to mixing different species and strains in published meta-analyses, but they did recommend further studies. In recent years, more probiotic trials have been done and this meta-analysis does present the outcomes separated by probiotic species and strain.

It was difficult to assess the most effective combination of probiotic strain and type of *H. pylori* eradication therapy, as most trials used a similar eradication therapy. In our review of 28 treatment arms, over 89% used triple therapy and the most common combination was amoxicillin, clarithromycin and omeprazole (36% of all triple therapies), followed by amoxicillin, clarithromycin and lansoprazole (18%). Eradication rates did not significantly differ by the type of eradication therapy and probiotic strain given, but the lack of variation and studies using the same eradication therapy and probiotic strain limited our analysis. It is also difficult to recommend the best daily dose and duration of a probiotic. Our subgroup analysis did not show a significant effect of daily dose, and doses used in trials with the same strain often had similar daily doses. Other meta-analyses that have investigated the effect of the dose and duration of the probiotic regime have not found a significant effect[88].

Most of the trials (89%) had sufficient follow-up

times (4-8 wk) to allow adverse events to occur, but 11% did not have any follow-up post-treatment. As only one trial followed patients for a prolonged time (one year), it is uncertain if the *H. pylori* eradication rates reported in the trials are transient or more permanent.

This systematic review has several strengths. We had specific outcomes selected *a* priori and the search strategy for this review was comprehensive including any relevant trials irrespective of language or publication status (*i.e.*, we included published data from meeting abstracts, obtained specific data from authors, and translated three non-English trials). Additional strengths of the review include its application of the GRADE criteria for each of the outcomes[31] and the rigorous evaluation of each of the subgroups (*i.e.*, same probiotic strain, probiotic dose, study population, and risk of bias) using the 33 criteria for assessing subgroup credibility[92]. The results of this meta-analysis may be generalizable to the global population, because we included a wide range of ages, countries and settings (inpatients and outpatients, adults and children were included). It should be noted however, that ethnicity and race data were not reported, nor were immunocompromised patients included in most of the trials, so the applicability of our results to these types of these populations is not known.

This review also has several limitations. While we did a more comprehensive search of the grey literature, we did not search all conference proceedings or dissertation abstracts. One of the main limitations for doing meta-analysis on probiotics is the limited number of probiotic strains that have data from multiple trials. Probiotic strain has been cited as the key indicator of efficacy for several diseases[23-25], but the limited number of trials on the same strain limits our ability draw robust conclusions on most of the strains used for all cited studies. We had to exclude 18 studies that only had one randomized controlled trial for a specific probiotic strain and, as a consequence, not all probiotic strains were included in this analysis. Another limitation is the changing designation of the probiotic strain over time. Older trials may refer to the same strain, but under a different strain type or the strain designation may not be provided in the published article. Other meta-analyses have grouped several strains of *L. casei* into one group (DG or DN114001 or Shirota), perhaps due to the lack of a current consensus on the taxonomy of these strains[93]. We did include one *L. acidophilus* study into our analysis, but it should be noted that the strain designation could not be determined retrospectively. This makes a systematic review challenging, as the authors must retrospectively find the matching strain designations as they change over time to include or exclude studies from specific probiotic strain groups.

Recommendations for future research include multiple randomized, controlled trials on the same probiotic strain, allowing confirmation of single clinical trial results. Improvements in the quality of study design should include complete description of the probiotic intervention (strain designation, daily dose, duration, source, *etc.*), use of treatment concealment (double blinding), calculating sample size *a* priori to power a sufficiently large study to detect significant results, use of intent-to-treat analysis to account for patient attrition effects, the collection of adverse event data and having sufficient follow-up time after the treatments are discontinued. In our meta-analysis, only four the trials had sufficient follow-up times (> 8 wk) to capture prolonged eradication of *H. pylori*. Future clinical trials need to incorporate sufficient follow-up times in their study protocols. None of the RCT in this meta-analysis reported any adverse events associated with probiotic use, which has been substantiated in other papers[94-96], but adverse event data should be collected and assessed for future studies.

In conclusion, our meta-analyses found only one strain of probiotic (*S. boulardii*, CNCM, I-745) is beneficial and safe in the eradication of *H. pylori* when combined with standard eradication therapy, and two strains of probiotics (*S. boulardii or L. rhamnosus* GG) decreased the adverse events of eradication therapy (including AAD), which may improve compliance in infected patients.

COMMENTS

Background

Helicobacter pylori (H. pylori) infections are a global problem and may lead to the development of a wide range of symptoms from dyspepsia to gastric cancer. The current therapy of multiple antibiotics and a proton pump inhibitor is associated with high frequencies of adverse events, which reduces compliance and increases treatment failure rates. The addition of probiotics to the standard treatments may assist in improving compliance, but the correct choice of probiotic strain is paramount.

Research frontiers

Over the years, many randomized controlled trials have been done to evaluate the efficacy of probiotics as adjunctive therapy for the eradication of *H. pylori* and/or development of adverse events, but previous reviews have been flawed or incomplete and may have inappropriately combined different types of probiotics into one group and thus could not achieve a comprehensive conclusion.

Innovations and breakthroughs

This comprehensive meta-analysis has used current guidelines for evaluating probiotic efficacy separately by the type of probiotic (only single strain probiotic trials grouped together) and evaluated each of three outcomes (*H. pylori* eradication, reducing any adverse events, reducing antibiotic-associated diarrhea) separately to determine which single probiotic strain may be efficious for each of the three outcomes. A total of 25 randomized controlled trials (with 28 treatment arms) of single strain probiotics were assessed. Of the six different probiotic strains evaluated, only two (*Saccharomyces boulardii* CNCM I-745 and *Lactobacillus rhamnosus* GG) were significantly associated with an improvement in at least one of the three outcomes.

Applications

These two probiotic strains can be used as adjunctive therapy to antibiotics used to treat *H. pylori* infections and may both improve compliance and reduce the development of adverse events, leading to better cure rates.

Terminology

Probiotics are living microbes (either fungal or bacterial), which when given at

appropriate doses, can affect the health status of the host.

Peer-review

The authors conducted a comprehensive literature review and data analysis on eradication of *H. pylori* by a single strain of probiotics. From literature collection to data analysis, it is all scientifically sound and the manuscript is well written.

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META-ANALYSIS

Addition of hip exercises to treatment of patellofemoral pain syndrome: A meta-analysis

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Abstract

AIM: To determine if the addition of hip-strengthening exercises decreases pain and improves function in patients with patellofemoral pain syndrome.

METHODS: The authors completed a systematic review searching eight databases (*i.e.*, PubMed, Cochrane, CINHAL, MEDLINE, SportsDiscus, EMBASE, APTA Hooked on Evidence, and PEDro). Two independent reviewers screened and excluded studies if they did not meet the following inclusion criteria: subjects had a primary diagnosis of patellofemoral pain syndrome (PFPS), intervention group included hip-strengthening exercises, control group included a traditional physical therapy intervention, study included outcome measures of pain and/or function, study used a randomized controlled trial design, PEDro score was $\geqslant$ 7, and study was published in a peer-reviewed journal. Primary outcome measures were subjective scales of pain and function. These measures were converted to standardized mean difference [effect size (ES)], and a random-effects model was used to calculate the overall ES.

RESULTS: Two hundred eighty-three studies were screened for inclusion in our meta-analysis. Nine studies were deemed suitable for data extraction and analysis. A total of 426 subjects were used in the nine studies. Overall, there was a significant positive effect of hip-strengthening exercises on measures of pain and function in subjects with PFPS (ES = 0.94, P = 0.00004). None of the individual studies had a negative ES, with study ES ranging from 0.35 to 2.59. Because of the high degree of between-study variance (I^2 = 76%; Q = 34.0, P < 0.001), subgroup meta-analyses and meta-regressions were performed. None of the potential moderator variables that were investigated (*e.g.*, outcome type, hip region targeted, duration of treatment) could explain a significant amount of the between-study variance in ES ($P \geqslant 0.23$).

CONCLUSION: Overall, the addition of hip-strengthening exercises to traditional physical therapy produced greater improvements in measures of pain and function.

Key words: Exercise therapy; Systematic review; Knee joint; Physical therapy modalities

Core tip: The most effective treatment to improve pain and function in patellofemoral pain syndrome is uncertain. We performed a systematic review and meta-analysis to determine if the addition of hip-strengthening exercises to traditional physical therapy interventions could effectively reduce pain and increase function in patients with patellofemoral pain syndrome. Our analysis indicates that the addition of hip-strengthening exercises provides a significant and relatively large additional reduction in pain and increase in function.

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INTRODUCTION

Patellofemoral pain syndrome (PFPS) is a prevalent lower-extremity disorder. PFPS can account for over 10% of physician office visits in an orthopedic setting, and account for 25%-40% of patients with knee pain and/or injury[1-4]. Women are twice as likely to be affected compared to men[3,5-7]. The etiology of PFPS has historically been attributed to abnormal tracking of the patella resulting from abnormal muscle forces, either weakness or tightness, and/or biomechanical factors (*e.g.,* Q angle, shallow trochlear groove) that alter the normal compressive and shear forces at the patellofemoral joint[1-8]. Lateral tracking of the patella can occur with an excessive Q angle at the knee, which is a measure of the angle of pull of the knee extensors in relation to the patellar tendon[2,3,8]. However, there is no consensus on PFPS's etiology.

Factors proximal to the patellofemoral joint are emerging as possible significant contributors to the cause of PFPS. There is a recent focus on the role of the hip abductor muscles in controlling the genu valgum angle at the knee during dynamic activity, finding that weakness of the hip abductors leads to an increased adduction/genu valgum moment with activity[8-10]. Weakness of the hip external rotators and extensors may also contribute to increased adduction and internal rotation of the lower leg with activity, thereby increasing biomechanical forces of shear and compression at the patellofemoral joint[10].

Traditional physical therapy interventions have focused on knee extensor strengthening, as well as bracing, taping, and modalities in treating patients with PFPS[2]. Often times, interventions focused strictly at the knee joint and knee extensors are not successful at decreasing a patient's pain complaint[2]. With the recent interest in the role of the proximal hip joint musculature contributing to PFPS, the objective of this study was to determine, utilizing a systematic review and meta-analysis, if the addition of hip-strengthening exercises to a traditional physical therapy intervention reduces pain and improves function in patients with PFPS more so than the traditional physical therapy intervention alone.

MATERIALS AND METHODS

Systematic review

We reviewed the research literature to identify studies that examined the effects of hip-strengthening exercises on pain and functional limitations in patients with PFPS. Our literature search began September 2013 and continued through October 2014. Databases including PubMed, Cochrane, CINHAL, MEDLINE, SportsDiscus, EMBASE, APTA Hooked on Evidence, and PEDro were searched electronically. The search terms included: "patellofemoral AND hip strength*" and MeSH terms (patellofemoral pain syndrome/rehabilitation AND hip) OR (patellofemoral pain syndrome/therapy AND hip).

Study inclusion and exclusion criteria: Two independent reviewers screened and excluded studies if they did not meet the following inclusion criteria: (1) study utilized subjects with a principal medical diagnosis of patellofemoral pain syndrome; (2) study included a treatment group performing hip-strengthening exercises in combination with or without a traditional physical therapy intervention; (3) study included a control group performing a traditional physical therapy intervention; (4) study named the muscles or muscle region targeted with exercises performed; (5) study measured pain or function as outcomes; (6) studies were randomized controlled trials and had a PEDro score greater than or equal to 7[11]; and (7) study was published in a peer-reviewed journal.

Selection of studies: Two hundred eighty-three studies were identified through the database searches and review of article reference lists. Of those, 126 studies were eliminated as duplicates among the different databases. Then, 135 studies were excluded on the basis of the title and/or review of the abstract. Twenty-two studies were fully evaluated via a careful review of the full text. On the basis of the inclusion and exclusion criteria, 13 studies were excluded leaving a total of nine studies to be included in the meta-analysis[12-20] (Figure 1).

Data extraction: For the meta-analysis, pain and function as reported by the numeric pain rating scale (NPRS), pain visual analog scale (VAS), Kujala anterior knee pain scale (AKPS), lower extremity functional scale (LEFS), and Womac pain rating scale data were extracted in the form of means, standard deviations, and sample sizes for the intervention (*i.e.,* group employing hip-strengthening exercises) and

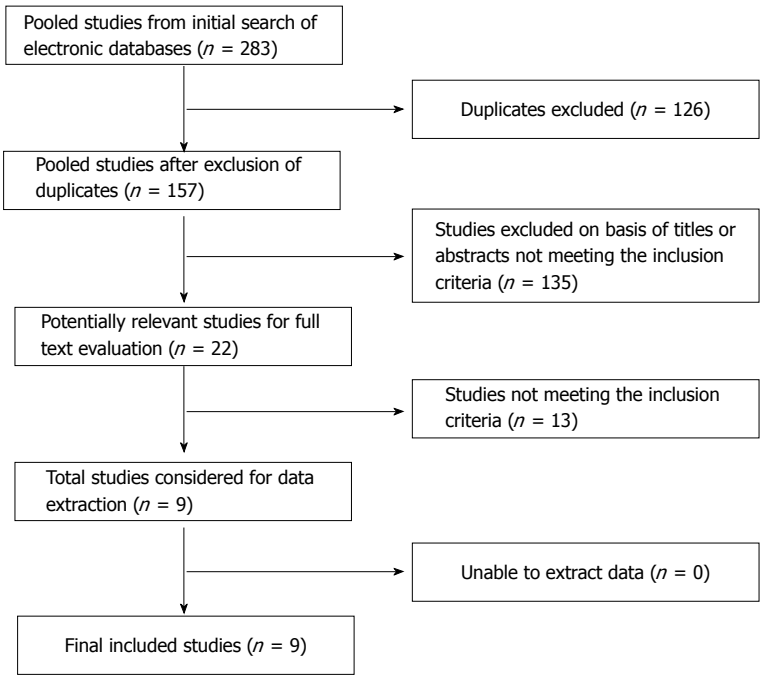

Figure 1 Flow diagram of the number of studies identified, the number excluded and the reasons for exclusions, and the final number of studies included in the systematic review and meta-analysis.

control groups. The number of sessions, region of hip targeted, total duration of treatment sessions, time for follow-up assessments, and subject descriptive measures were also extracted from the studies. The region of the hip targeted with exercise was extracted from the studies, and categorized as posterolateral if the exercises were isolated to the posterolateral hip muscles or general if the exercises involved the major thigh and muscles that cross the hip joint. The time for follow-up was extracted from the studies and categorized as immediate follow-up if outcome assessments were done when the intervention ceased or as long-term for any assessment performed 3 or more months after the intervention ceased. Exercise intensity was extracted for all studies but was not found to be usable because of the variability among studies in how intensity was expressed or because it varied within and between sessions in some studies but not in others.

Meta-analysis

The extracted pain and functional measures data were converted to a standard format, *i.e.*, standardized mean difference, which will be referred to as an effect size (ES) from this point on. Meta-analyses were run using the random-effects model that accounts for true between-study variation in effects as well as random error within each study. A random-effects model was employed for this analysis because the nine studies used dissimilar experimental designs and/or procedures[21]. Between-study variance was assessed using the Q value and I^2. Because substantial between-study variance was detected, we sought to determine the role that different experimental factors might have in explaining this variance. These factors can be treated as potential moderator variables. Meta-regressions (using a methods of moment model) or subgroup meta-analyses were used to probe the following potential moderator variables: region of hip targeted during the hip exercises, number of exercise sessions, duration in weeks of exercise intervention, control group type, outcome type, and time of follow-up. Subgroup meta-analyses and meta-regressions were used for analysis of categorical and continuous variables, respectively. In studies with more than one experimental factor level being evaluated (*e.g.,* a study using both pain and function outcomes in the subgroup meta-analysis evaluating the effect of outcome type), an ES was calculated for each level and was treated as if it originated from an independent study.

Meta-analyses were conducted using comprehensive meta-analysis software (version 2.2; Biostat Inc., Englewood, NJ). An α level of 0.05 was used in all analyses. Effect sizes of 0.2, 0.5, and 0.8 were considered to be small, moderate and large respectively[22]. The possible effect of publication bias on the meta-analysis was assessed by visual assessment of a funnel plot and using Duval and Tweedie's trim and fill correction.

RESULTS

Description of included studies

In total, nine studies were included in the meta-analysis examining the effect of hip-strengthening exercises on pain and function in persons with PFPS. The characteristics of these studies are summarized in Table 1. All nine studies were published in peer-reviewed journals and used a randomized controlled trial design. Subjects were randomly assigned to the two groups, *i.e.,* one receiving a traditional intervention and one receiving traditional intervention plus hip exercises. Therapy providers were not blinded

Table 1 Characteristics of the nine studies examining the effects of hip strengthening exercises on pain and function in patients with patellofemoral pain syndrome

Ref.	Subject information	Subject mean age (min-max)	Hip region targeted	Outcome measured	Time to follow-up (mo)	Number of exercise sessions	Exercise duration (wk)	PEDro quality score (0-11)
Dolak *et al*[12]	33 women	25.5 (16-35)	Posterolateral	Pain VAS and LEFS	0, 1[1], 2[1]	12	4	7
Fukuda *et al*[14]	41 women	25 (20-40)	Posterolateral	NPRS, Kujala AKPS, LEFS	0	12	4	9
Fukuda *et al*[13]	49 women	22.5 (20-40)	Posterolateral	NPRS, Kujala AKPS, LEFS	3, 6, 12	12	4	9
Herrington *et al*[15]	30 men	26.9 (18-35)	General	Pain VAS, Kujala AKPS	0	18	6	9
Ismail *et al*[16]	32 (9 men, 23 women)	21 (18-30)	Posterolateral	Pain VAS, Kujala AKPS	0	18	6	8
Khayambashi *et al*[17]	36 (18 men, 18 women)	27.8 (12-44)	Posterolateral	Pain VAS, Womac	0, 6	24	8	7
Nakagawa *et al*[18]	14 (4 men, 10 women)	23.6 (17-40)	Posterolateral	Pain VAS	0	30	6	10
van Linschoten *et al*[19]	131 (47 men, 84 women)	24 (14-40)	General	Pain VAS, Kujala AKPS	0, 9	84	12	7
Witvrouw *et al*[20]	60 (20 men, 40 women)	20.3 (14-33)	General	Pain VAS, Kujala AKPS	3, 60[1]	15	5	8

[1]Data for this time point not included in analysis due to lack of baseline data matched to subjects in that time to follow-up and/or a change in the intervention regimen that did not meet our criteria. VAS: Visual analog scale; NPRS: Numeric pain rating scale; LEFS: Lower extremity functional scale; AKPS: Anterior knee pain scale.

to which group they were assigned to. Assessors administering the outcome assessments (*i.e.,* Pain VAS, NPRS, LEFS, and Kujala AKPS) were blinded to the groups that the subjects were assigned to; however, subjects completed these questionnaires and were aware of the group they were assigned to. All but one study included measures of both pain and function; the one study included only a measure of pain[18]. Seven of the studies measured outcomes immediately after completing the intervention[12,14-19], while two studies did not make assessments of pain and function until at least 3 mo post intervention[13,20]. Dolak *et al*[12] made a follow-up assessment at 1 and 2 mo post intervention; however, this data was not included in the analysis because the exercise regimen changed after post-treatment and did not meet our inclusion criteria. Witvrouw *et al*[20] made a follow-up assessment at 5 years post intervention; however, these data were not included in the analysis because baseline measures were not available for the subjects who reported for the 5-year follow-up and subjects were inconsistent in adhering to their exercise regimen during this period. Six studies specified hip exercises as targeting the posterolateral musculature of the hip, such as hip abduction, hip lateral rotation and hip extension[12-14,16-18], while three studies' hip exercises were considered general to the hip musculature[15,19,20]. A total of 426 subjects were used in the nine studies. Subject gender in the studies was generally a mixture of men and women but one study used men only[15] and three studies used women only[12-14]. The duration of intervention varied among studies from 4 to 12 wk, with total number of treatment sessions ranging from 12 to 84.

Meta-analysis on pain and function outcomes

When combining all outcome types and times for follow-up, meta-analysis of all nine studies yielded a statistically significant and large effect size (ES = 0.94, P = 0.00004), indicating that patients with PFPS performing hip exercises in addition to traditional physical therapy interventions reported less pain and increased function than control subjects receiving traditional interventions (Figure 2). None of the individual studies had a negative ES, with the standardized mean difference ranging from 0.35 to 2.59. No one study was found to dominate the calculation of the overall ES. Fukuda *et al*[13] 2012 had the single largest effect on the overall ES but even if it was removed from the analysis, the overall ES was still moderate-to-large and statistically significant (ES = 0.67, P = 0.000001). Publication bias also did not appear to affect the overall ES. We did not observe any overt asymmetry in the funnel plot of standard error versus study ES. Furthermore, when the Duval and Tweedie's trim and fill adjustment was applied to correct for potential publication bias, no studies were trimmed and thus the procedure made no adjustment to the overall ES.

The two assessments of variation in ES among the studies indicate that the variation is both large (I^2 = 76%) and statistically significant (Q = 34.0, df = 8, P < 0.001). Because of this variability, subgroup meta-analyses and meta-regressions were used to probe possible roles for six experimental factors that might help to explain ES variation among the nine

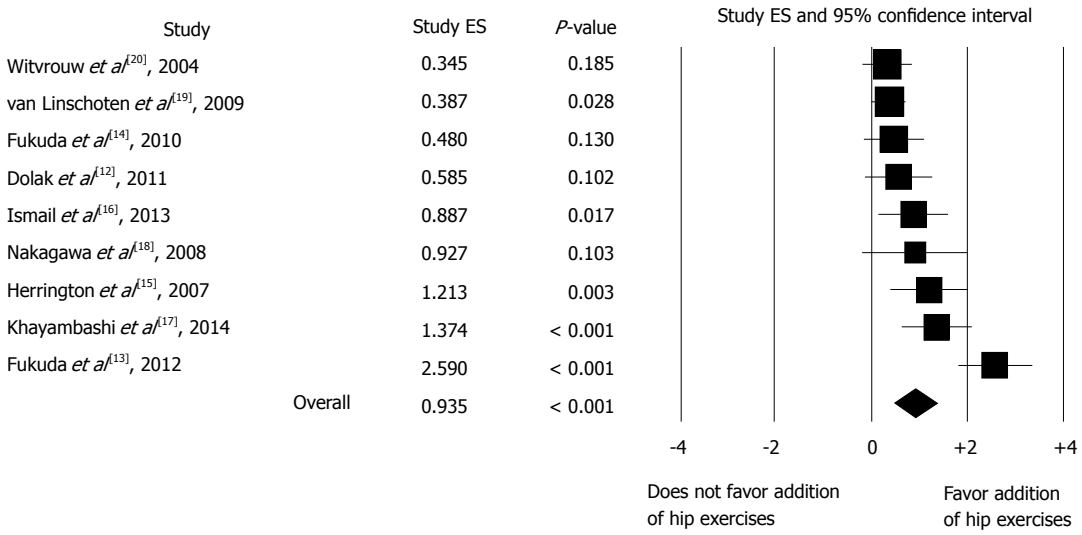

Figure 2 Forest plot of effect sizes from studies that assessed the effect of hip strengthening exercises on pain and function in patients with patellofemoral pain syndrome. A square represents the effect size for a given study with the size of the square being proportional to the weighting of that study in the meta-analysis. A horizontal line indicates the 95%CI for an effect. Studies are arranged from lowest to highest effect size. The diamond at the bottom represents the overall effect size calculated using a random-effects model. The width of the diamond represents the 95%CI for the overall effect size.

studies. Table 2 summarizes the findings of those analyses. None of the experimental factors were able to significantly account for any ES variation. Subgroup analysis of outcome type, time of follow-up, control group type, and hip region targeted with exercise indicated that these variables could not explain a significant amount of the between-study ES variation ($P \geqslant 0.23$). Using meta-regression, it was determined that exercise duration in number of weeks and the total number of treatment sessions also could not explain a significant amount of the between-study ES variation ($P \geqslant 0.38$).

DISCUSSION

The main finding of this study is that in persons diagnosed with patellofemoral pain syndrome the addition of hip-strengthening exercises to traditional physical therapy produced greater improvements in measures of pain and function than traditional therapy alone. Given this finding, developing a targeted program to strengthen both the hip and knee musculature as opposed to alternatives such as strengthening only the knee extensors may lead to fewer number of physical therapy and doctor visits and overall quicker recovery times. Interestingly, the number of exercise sessions and/or number of weeks of exercise intervention did not appear to affect the variation between studies in the effectiveness of the hip-strengthening exercises.

There are several potential limitations of our systematic review and meta-analysis, as well as some methodological concerns with the underlying studies themselves. One possible limitation of our systematic review was publication bias. Publication bias occurs when published research is systematically unrepresentative of the total population of studies[21]. Studies with non-significant and/or negative findings are less likely to be published, and this may influence the overall ES in a meta-analysis that is based largely on published studies. Publication bias was assessed in our review by examination of the funnel plot. Additionally, the Duval and Tweedie's trim and fill adjustment was applied but there was no correction to the overall ES. But because of the relatively few studies, the sensitivity of these analyses could be lacking. Furthermore, we did not rigorously examine the grey literature for unpublished studies.

A second potential limitation of our analysis was the inability to explain the substantial between-study variance in ES. Subgroup analyses and meta-regressions did not identify any experimental factors that could help explain this variance. Many of these analyses probably did not have adequate statistical power because of the limited number of studies in the review and because some subgroups had as few as three studies in them. We tried to assess the ability of gender to explain the between-study variance in ES but could not run a subgroup analysis on gender because there was only one study that used only male subjects. Another concern of the systematic review and meta-analysis is that the exercises performed in each study were categorized by the region of the thigh the exercises targeted (*i.e.*, knee extensors, general hip, posterolateral hip) *vs* listing each specific exercise performed. Thus, we were not able to assess how the performance of specific exercises might explain the between-study variance in ES and enable us to hypothesize a particular exercise to be more effective in reducing pain and improving function in patellofemoral pain syndrome. We also were not able to assess if the exercise intensity for the interventions might explain the between-study variance in ES.

A third potential limitation of our analysis is the

Table 2 Summary of subgroup meta-analyses and meta-regression analyses examining potential moderator variables that might explain the variation in effect size among studies

Moderator variable	Comparison (or slope for continuous variables)	*P* value
Outcome type	Function (n = 8, ES = 0.92) *vs* Pain (n = 9, ES = 0.95)	0.95
Time of follow-up	Immediate (n = 7, ES = 0.79) *vs* Long term (n = 4, ES = 1.111)	0.44
Control group type	Knee extensor strengthening only (n = 6, ES = 1.15) *vs* Knee extensor strengthening plus other (n = 3, ES = 0.56)	0.24
Hip region targeted with exercise	General hip (n = 3, ES = 0.60) *vs* Posterolateral hip (n = 6, ES = 1.13)	0.23
Number of exercise sessions	-0.009/session	0.38
Number of weeks of exercise	-0.066/wk	0.48

ES: Effect size.

inability to completely blind the subjects and therapy providers within the individual studies. All studies are randomized control trials with random assignment of subjects to groups. Subjects that have basic knowledge of anatomy and exercise would likely be aware of which group they were assigned to. While assessors administering the outcome assessment tools (*i.e.,* Pain VAS, NPRS, LEFS, and Kujala AKPS) were blinded to subject group assignment, the subjects themselves completed the outcome tools which consists of questionnaires. Whether an assessor is blinded or not should not affect how a subject completes these forms.

This study's findings provide justification for future research. All study ES including the overall ES were positive, suggesting that despite the large variation in experimental design among studies, the addition of hip strengthening to traditional physical therapy interventions is beneficial in reducing pain and function in patellofemoral pain syndrome when compared to traditional knee-focused interventions alone. Future research examining whether hip-strengthening exercises are equally effective in men and women is important to know, especially when considering that women are more frequently diagnosed with patellofemoral pain syndrome. It would also be helpful, with a larger number of studies, to be able to identify individual hip exercises that are more beneficial than others in decreasing pain and improving function.

COMMENTS

Background

Patellofemoral pain syndrome (PFPS), is a common disorder of the knee. There is no consensus on the etiology of PFPS, however there is an emerging focus on the contribution of proximal structures, *i.e.,* the hip, on PFPS. Traditional therapeutic exercises performed to address PFPS focus on strengthening the knee extensor muscles.

Research frontiers

Interventions targeting the more proximal segment, the hip, in treating PFPS are becoming more of a focus in rehabilitation than targeting the knee extensor muscles alone.

Innovations and breakthroughs

Previous systematic reviews that looked at PFPS only performed review of the literature. The present study included more high quality studies and performed a meta-analysis to quantitatively assess the effect of hip exercises on PFPS compared to traditional interventions.

Applications

The present study suggests that the addition of hip strengthening exercises to traditional therapy improves pain and function in patients with PFPS.

Terminology

Patellofemoral pain syndrome is a diagnosis characterized by anterior knee pain surrounding the patella.

Peer-review

The authors present a well written manuscript with a sound conclusion.

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Red meat intake and the risk of endometrial cancer: Meta-analysis of observational studies

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Abstract

AIM: To evaluate whether red meat intake is related to the risk of endometrial cancer (EC) using meta-analysis.

METHODS: We searched PubMed, EMBASE, and the Cochrane Library up to June 2013, using common keywords related to red meat and EC. Case-control studies and cohort studies comparing the risk of endometrial cancer among categories by the amount of intake were included. Eleven case-control studies and five cohort studies met our criteria. We performed a conventional and a dose-response meta-analysis of case-control studies using the DerSimonian-Laird method for random-effects. For cohort studies we performed a conventional meta-analysis. Publication bias was evaluated using Egger's test.

RESULTS: In the meta-analysis of 11 case-control studies including 5419 cases and 12654 controls, higher red meat consumption was associated with an increased risk of EC [summary relative risk (SRR) = 1.43, 95%CI: 1.15-1.79; I^2 = 73.3% comparing extreme intake categories). In a dose-response analysis, for red meat intake of 100 g/d, SRR was 1.84 (95%CI: 1.64-2.05). In contrast, in the meta-analysis of five prospective studies including a total of 2549 cases among 247746 participants, no significant association between red meat intake and EC risk (SRR = 0.97, 95%CI: 0.85-1.11; I^2 = 4.9% comparing extreme intake categories) was observed.

CONCLUSION: Our meta-analysis found a significant

linear association between red meat intake and EC risk based on case-control studies but this was not confirmed in prospective studies.

Key words: Red meat; Endometrial cancer; Dose-response; Meta-analysis; Observational studies

Core tip: By conducting a dose-response meta-analysis, we found a significant linear association between red meat intake and endometrial cancer risk based on case-control studies. However this association was not confirmed in prospective studies. In our paper, we argue that those findings are attributable to methodological difference between retrospective case-control studies and prospective studies.

Ju W, Keum N, Lee DH, Kim YH, Kim SC, Ding EL, Cho E. Red meat intake and the risk of endometrial cancer: Meta-analysis of observational studies. *World J Meta-Anal* 2015; 3(2): 125-132 Available from: URL: http://www.wjgnet.com/2308-3840/full/v3/i2/125.htm DOI: http://dx.doi.org/10.13105/wjma.v3.i2.125

INTRODUCTION

Endometrial cancer (EC) is estimated to be the fourth most common cancer in females in the United States in 2013[1]. Risk factors for EC include obesity, postmenopausal hormone replacement therapy (HRT), type Ⅱ diabetes, tamoxifen use, and conditions related to unopposed estrogen such as chronic anovulation and estrogen-only HRT[2-4]. In particular, obesity measured by body-mass index (BMI) has been a well-established risk factor present in almost 50% of women with EC[5]. Recently, red meat intake has received an increasing attention as a potential risk factor for EC[6-8].

An harmful effect of red meat intake has been most studied with colorectal cancer (CRC)[9]. A meta-analysis of 26 cohort studies found an approximately 20% increased risk of colorectal cancer with higher red meat intake[10]. Given that CRC and EC share similar risk factors such as obesity, diabetes, and low physical activity, it has been hypothesized that high red meat intake may increase the risk of EC. This hypothesis is further supported by several mechanisms. Heterocyclic amines generated by overcooking or N-nitroso compounds from proteins have been suggested to act as carcinogens[11]. Iron component in red meat may increase the risk of EC by damaging DNA through oxidative stress[12].

While considerable observational studies have been conducted to examine the effect of red meat intake on EC risk, the epidemiologic relationship remains inconclusive. World Cancer Research Fund (WCRF) panel concluded that there was limited evidence suggesting red meat as a risk factor for EC[13]. While past meta-analysis suggested evidence for a significant inverse association (SRR = 1.59, 95%CI: 1.24-2.05; I^2 = 50.2%, comparing extreme intake categories) and quantified that 100 g/d intake was significantly associated with an approximately 60% increased risk of EC (SRR = 1.6, 95%CI: 1.26-2.03)[14], this meta-analysis included only one prospective study[15]. Several prospective studies have been published afterward[6,7,16,17]. Therefore, we aim to conduct an up-to date dose-response meta-analysis of red meat consumption and the risk of EC based on case-control studies and prospective cohort studies.

MATERIALS AND METHODS

Literature search

We searched PubMed, EMBASE, and the Cochrane Library up to June 2013, using common keywords related to red meat and EC. The keywords were combined as follows: "meat products" as a Medical Subject Headings (MeSH) term or "animal protein" or "red meat" or "meat" and "endometrial neoplasm" as a MeSH term or "uterine cancer" or "corpus cancer" or "endometrial carcinoma" or "uterine carcinoma" or "corpus carcinoma" We also reviewed the bibliographies of relevant articles to locate additional publications. The language of publication was restricted to English.

Selection criteria

We included observational studies that met all of the following inclusion criteria: studies with human subjects, measured outcomes with pathologic confirmation, RR(s) of red meat intake for EC, and statistical information sufficient enough to restore CI(s) for RR(s). If data were duplicated or shared in more than one study, the most comprehensive study with the greatest number of cases was included in the analysis.

Selection of relevant studies

Based on the pre-determined selection criteria, two of the authors (Ju W, Keum N) independently reviewed all studies retrieved from the databases and bibliographies. Two authors screened titles for initial selection and reviewed abstracts/tables of the initially selected articles to identify relevant studies. The reference lists of articles included in our analysis and studies included in the previous meta-analyses were also reviewed for additional papers. Inconsistency between researchers was resolved through discussion based on full articles or in consultation with the third author (Lee DH).

Data extraction

From each study, the following information was extracted: author, year of publication, study design, cohort name, country of study, study period, age range at baseline, types of exposure (red meat, all type of meat except fish), intake range (g/d, g/wk, servings/wk recent), the most fully adjusted measures of association [odds ratio

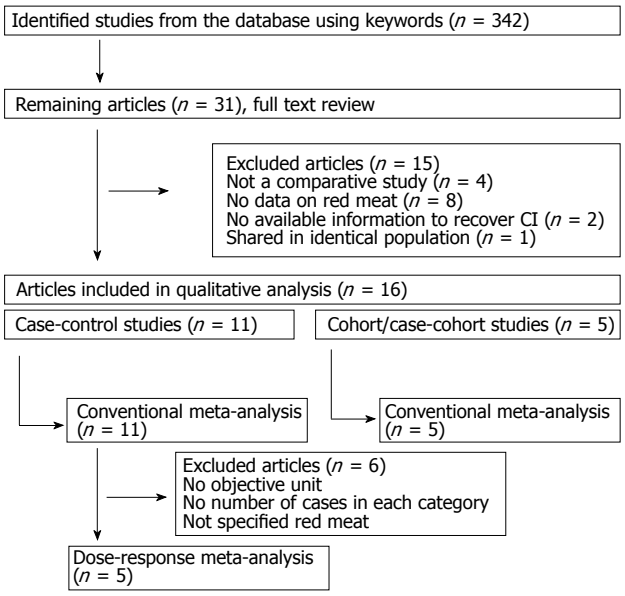

Figure 1 Flow diagram for identification of relevant studies.

(OR), rate ratio(RR), or hazard ratio (HR)], 95%CI, the number of cases, and total number of participants or person-time in each exposure categories. For the quality assessment of each study, exclusion criteria (hysterectomy, prior endometrial cancer), use of validated questionnaire, variables adjusted for confounding were extracted. For case-control studies, information on the types of controls (population *vs* hospital) was additionally extracted.

Statistical analysis

Both conventional and linear dose-response meta-analyses were performed. We assumed a linear dose-response relation in two points. First the previous studies[18,19] with CRC showed linear dose-response association up to 100 g/d intake. Second even in non-linearity model, over 100 g/d did not showed a reduced risk but a blunted slope of increasing risk, which means additional harmful effect of the same direction. In the conventional meta-analysis, random effects model was used to calculate the summary OR for the highest *vs* lowest intake and red meat and the 95%CI. Heterogeneity was assessed using Q test and I^2. Potential sources of heterogeneity were explored using meta-regression based on a priori selected variables. The quality of respective studies was evaluated by performing meta-regression in relation to proper definition of exclusion criteria, types of controls, use of validated questionnaire, type of exposure, adjustment for at least BMI, parity, and menopausal status. Potential publication bias was visually checked using funnel plot and statistically assessed with Egger's regression asymmetry test. Sensitivity analyses were performed by omitting each study at a time.

For the dose-response meta-analysis, a subset of studies included in the conventional meta-analysis was used if they satisfy the following criteria: availability of red meat intake in objectively quantifiable units; having at least three categories of red meat intake including the reference category; availability of number of cases, either number of participants or person-time, and 95%CI for each exposure category, Aggregate method assuming random effects model was used to calculate the SRR of EC associated with 100 g/d intake of red meat and 95%CI. For every study, the mean level of red meat intake in each category was assigned to the corresponding measure of association. In order to calculate the category-specific mean intake for the open-ended highest category, the length of the adjacent interval was assumed; for the open-ended lowest category, 0 g/d was set as a lower limit.

All statistical analyses were conducted using STATA 12 software package (StataCorp, College Station, TX) and based on 2-tailed α set at $P \leqslant 0.05$ for statistical significance. In dose-response meta-analysis we used "Generalized Least Squares" in STATA, which considers the correlation among exposure categories by approximating covariance with GL method.

RESULTS

Figure 1 shows how we identified relevant studies. Initial search identified a total of 342 articles, of which 311studies were excluded for not satisfying the pre-determined selection criteria. We reviewed the full texts of the remaining 31 articles and further excluded 15 articles for the following reasons: not a comparative study (n = 4); no data on red meat (n = 8); no information to recover CI (n = 2); shared in identical population (n = 1). Finally, a total of 16 studies (11 case-control studies, 5 prospective studies) were included in our meta-analyses[6,7,15,16,20-30]. and their characteristics are summarized in Tables 1 and 2, respectively.

Retrospective case-control studies

The 11 case-control studies included a total of 5419 cases and 12654 controls. The year of publication of the included studies ranged between 1993 and 2009. The countries where the studies were conducted were as follows: United States (n = 5), China (n = 2), Greece (n = 1), Italy (n = 1), Sweden (n = 1), and Switzerland (n = 1).

Both conventional and linear dose-response meta-analyses were performed.

Conventional meta-analysis: The SRR comparing the "highest" with the "lowest" categories of red meat intake was 1.43 (95%CI: 1.15-1.79), with considerable heterogeneity (I^2 = 73.3%, $P_{heterogeneity}$ < 0.001), which are shown in Figure 2. In this random-effects meta-analysis, z-value for the overall effect was 3.18 and P-value for test of effect size was 0.001.

None of a priori selected factors such as type of exposure (red meat *vs* all type of meat) and publication year, and BMI adjustment was a significant source of heterogeneity. Publication bias was not evident with Funnel plot showing a symmetric dispersion of studies

Table 1 Summary of case-control studies of red meat consumption and endometrial cancer

Ref.	Location	Study base, subjects	Nutrient	Measurement (unit)	Reference year	Adjustment factors	OR (95%CI)	Meta-analysis	
								Con-ventional	Dose-response
Shu *et al*[20]	China	Population; 268 cases, 278 controls	Red meat	1 liang (50 g)	10 yr prior to interview	Age, number of pregnancies, BMI, caloric intake	2.5	√	√
Potischman *et al*[21]	United States	Population; 399 cases, 296 controls	Red meat	Times/wk	Past few years	Age, BMI, estrogen use, oral contraceptive, number of births, current smoking, education, total calories	1.3 (0.8-2.4)	√	√
Levi *et al*[22]	Swiss	Hospital; 274 cases, 572 controls	Beef	Subjective score	Year before the occurrence of symptoms	Study center, age	2.26 (1.57-3.24)	√	
Goodman *et al*[23]	United States (Hawaii)	Population; 332 cases, 511 controls	Red meat	g	Year prior to diagnosis	Age, ethnicity, pregnancy history, oral contraceptive, diabetes, BMI, total calories	2 (1.1-3.7)	√	√
McCann *et al*[24]	United States	Population; 232 cases, 639 controls	Red meat	Times/mo	2 yr prior to interview	Age, education, BMI, diabetes, hypertension, smoking, age at menarche, parity, oral contraceptive, menopausal status, estrogen	0.8 (0.5-1.4)	√	√
Tavani *et al*[25]	Italy	Hospital; 750 cases, 4770 controls	Red meat	Portions/wk	2 yr preceding diagnosis	Age, year of recruitment, education, smoking, alcohol, fat, fruit, vegetables	1.5 (1.2-1.8)	√	√
Littman *et al*[26]	United States	Population; 679 cases, 944 controls	All meat	Servings/d	5 yr prior to diagnosis	Age, residence, total energy intake, unopposed estrogen, smoking, BMI	1 (0.75-1.4)	√	
Terry *et al*[27]	Sweden	Hospital; 709 cases, 2887 controls	All meat	Quartile	1 yr before diagnosis	Age, BMI, smoking, physical activity, diabetes, fatty fish consumption, total food consumption	1.3 (1.0-1.8)	√	
Dalvi *et al*[28]	United States	Population; 488 cases, 461 controls	Western diet	Quintile	1 yr preceding diagnosis	Age, race, age at menarche, oral contraceptive, parity, daily calorie intake, physical activity, menopause, hormone therapy, BMI	1.5 (0.77-3.0)	√	
Xu *et al*[29]	China	Population; 1204 cases, 1212 controls	Red meat	1 liang (50 g)	Past 5 yr	Age, menopause, diabetes, alcohol, BMI, physical activity, total energy intake, other meat	1.3 (1.0-1.8)	√	√
Petridou *et al*[30]	Greece	Hospital 84 cases 84 controls	All meat	Frequency/mo	1 yr preceding onset of disease	Education, BMI, pregnancy, total energy intake	0.78 (0.53-1.16)	√	

OR: Odds ratio; BMI: Body mass index.

(Figure 3A) and with Egger's test non-significant (P = 0.911, intercept: -0.20, 95%CI: -4.21-3.80).

Meta-regression for assessing the quality of individual studies showed that methodological components such as exclusion criteria, types of controls, validation of dietary questionnaire, and confounding adjustment did not significantly modify the relationship between red meat intake and EC.

Dose-response meta-analysis: Six out of the eleven studies were eligible for the dose-response meta-analysis, including a total of 3364 cases and 10916 controls. Figure 4 illustrates a significant linear dose-response relationship between red meat intake and EC risk. For each 100 g/d increase of red meat intake, SRR was 1.84 (95%CI: 1.64-2.05), with no significant evidence for heterogeneity (I^2 = 21.7%, $P_{heterogeneity}$ = 0.21). In this dose-response meta-analysis, z-value for the overall effect was 10.75 and P-value for test of effect size was less than 0.001.

Prospective observational studies

Four cohort studies and one case-cohort study were identified and included a total 549 cases among 247746 participants. Only conventional meta-analysis was conducted because the five studies did not provide all the necessary information needed for linear dose-response meta-analysis. The SRR of EC for the highest *vs* lowest category of red meat intake was 0.97 (95%CI: 0.85-1.11), with little heterogeneity (I^2 = 4.9%, $P_{heterogeneity}$ = 0.38) (Figure 5). In this random-effects meta-analysis, z-value for the overall effect was 0.44 and P-value for test of effect size was 0.66. No publication bias was indicated by Funnel plot inspection (Figure 3B) and Egger's test (P = 0.142, intercept = 1.61, 95%CI: -0.98-4.20).

Table 2 Summary of cohort, case-cohort studies of red meat consumption and endometrial cancer

Ref.	Location	Cohort	Study design	Population	Nutrient	Intake unit	Reference year	Adjustment factors	RR (95%CI)
Zheng *et al*[15]	United States	Iowa Women's Health Study	Cohort	23070 total 216 cases	Total meat	g/d	Baseline	Age, age at menopause, parity, hormone therapy, total energy intake	1.1
Kabat *et al*[16]	Canada	National Breast Screening Study	Cohort	426 cases 33722 non-cases	Red meat	g/d	Baseline	Age, BMI, menopause, parity, age at menarche, estrogen use, oral contraceptive, total calories, raw vegetable, alcohol intake, physical activity, education	0.86 (0.61-1.22)
van Lonkhuijzen *et al*[6]	Canada	Canadian Study of Diet, Lifestyle, and Health	Case-cohort	56837 total 221 cases 3697 non-cases	Red meat	g/d	Baseline	Age, BMI, age at menarche, number of live birhts, breastfeeding, oral contraceptive, exercise, average calorie, vegetable intake, postmenopausal status, hormone therapy	1.62 (0.86-3.08)
Genkinger *et al*[7]	Sweden	Swedish Mammography Cohort	Cohort	60895 total 720 cases	Red meat	g/wk	Baseline	Age, energy, BMI, parity, and education	1.06 (0.68-1.66)
Arem *et al*[17]	United States	NIH-AARP Diet and Health Study	Cohort	72796 total 966 cases	Red meat	g/1000 kcal	Baseline	Age, BMI, smoking, total energy intake, age at menarche, age at first child's birth, parity, age at menopause, hormone therapy, oral contraceptive, diabetes, physical activity	0.91 (0.77-1.08)

RR: Risk ratio.

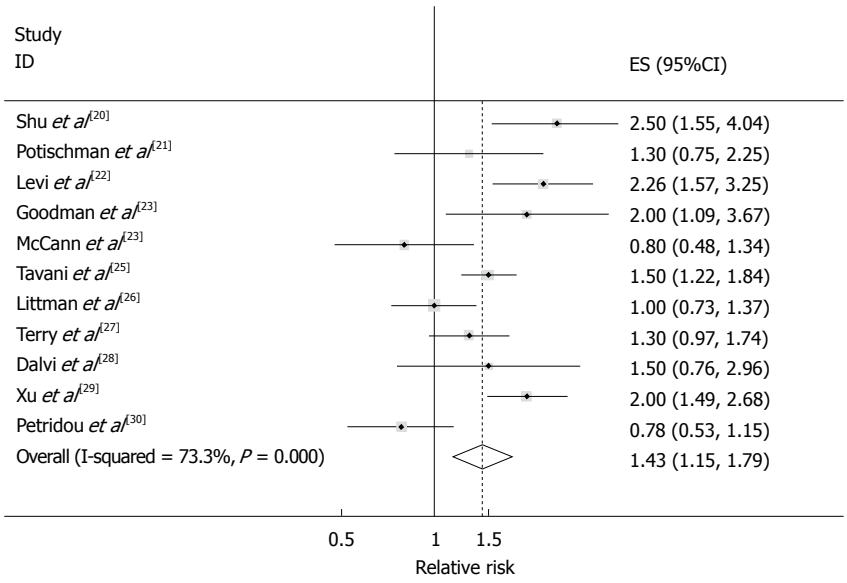

Figure 2 Random-effects meta-analysis of red meat intake and endometrial cancer risk in case-control studies, which shows I^2 = 73.3%, $P_{heterogeneity}$ < 0.000.

DISCUSSION

In this conventional and dose-response meta-analysis of observational studies, we found inconsistent results with retrospective case-control studies suggesting a significant increase in EC risk approximately by 84% associated with 100 g/d intake of red meat while with prospective observational studies indicating no such association.

Conventional meta-analysis which dichotomizes continuous exposures as highest *vs* lowest categories and collapses intake categories regardless of intake level ignores absolute intake and is not optimal to elucidate a dose-response relationship between dietary intake and disease outcomes. This approach may be particularly problematic in populations with wide intake range where cutoffs of intake categories are substantially different. A highest dosage in one study could be a reference dosage in another study, which means that the SRR from conventional meta-analysis

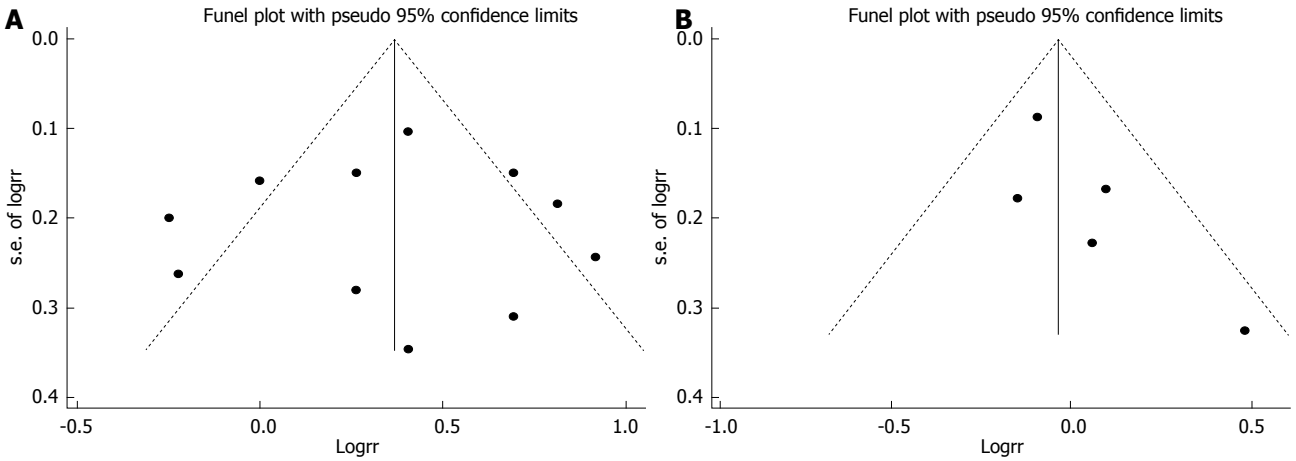

Figure 3 Funnel plot of eleven case-control studies (A) and five cohort studies (B) which were included in conventional meta-analysis.

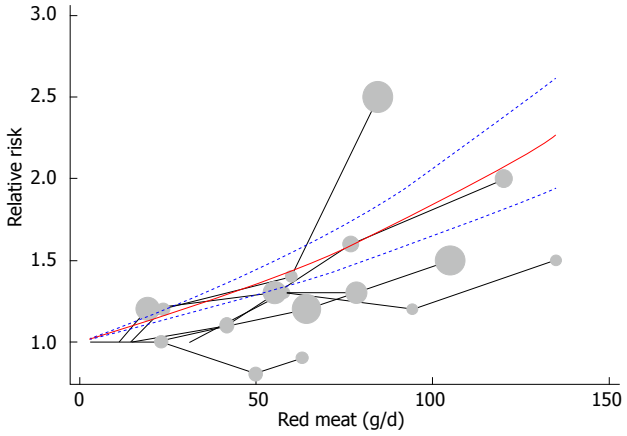

Figure 4 Dose-response spaghetti plot of red meat intake and endometrial cancer risk in case-control studies with pooled OR (solid line) and 95%CI (dotted lines).

might not give proper weights proportional to the dose-related risk. Dose-response meta-analysis has its practical importance than conventional meta-analysis of comparing extreme intake groups by providing summary estimates per absolute amount of intake, which can be easily incorporated in cancer prevention strategy and dietary policy.

Our findings based on 11 retrospective case-control studies are consistent with results from the previous meta-analysis of 7 case-control studies. The SRRs of 1.43 (95%CI: 1.15-1.79) for the highest *vs* lowest category of red meat intake and of 1.84 (95%CI: 1.64-2.05) per 100 g/d increment in the intake were similar to corresponding SRRs of 1.59 (95%CI: 1.24-2.05) and 1.60 (95%CI: 1.26-2.03) in the previous meta-analysis[14].

Despite those cumulative evidences arising from case-control studies suggests positive associations, most prospective studies have not supported an increased risk of EC associated with red meat intake. In 2000, Trichopoulou *et al*[31] summarized the nutritional etiology of various forms of cancer in their review, but did not find an evidence for a positive relationship between red meat intake and EC. In 2007, the Panel of WCRF concluded that although evidence for harmful effects of red meat and processed meat on EC risk was stronger than it had been in the mid-1990s, overall evidence remained suggestive, at most[13]. The most recent meta-analysis performed by Bandera *et al*[14] did not reported a pooled RR for cohort studies because they included only one cohort study by Zheng *et al*[15], which found no significant association (OR, 1.10, 95%CI: 0.79-1.52). Our updated meta-analysis of five prospective studies still suggests that red meat intake was not significantly associated with the risk of EC (SRR, 0.97, 95%CI: 0.85-1.11; I^2 = 4.9%). The discrepant results between retrospective case-control and prospective observational studies can be partially explained by several issues related to the measurement of red meat intake. First, retrospective *vs* prospective nature of measurement is an important consideration. In retrospective case-control studies, red meat intake was assessed after diagnosis of EC and thus, participants' knowledge about disease status could lead to differential measurement error. For instance, since cases are more sensitive to their dietary intake than controls in general, it is entirely possible that cases over-report their red meat intake, which could lead to the observed positive association between red meat intake and EC risk. In contrast, in prospective studies, red meat intake was assessed prior to the diagnosis of EC and thus, measurement errors are likely to be random with respect to disease status. Random measurement error of dichotomous exposure mostly attenuates a measure of association toward the null and thus, could partially account for the null association observed in our meta-analysis of cohort studies.

Second, difference in reference year for exposure measurement relates to differential assumption regarding etiologic window of red meat intake in affecting EC risk, which could lead to inconsistent results. In case-control

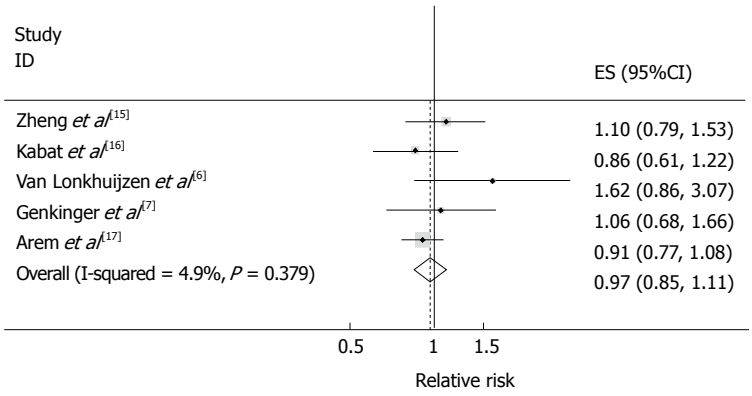

Figure 5 Random-effects meta-analysis of red meat intake and endometrial cancer risk in prospective studies.

studies, participants were asked to recall red meat intake during 1-5 years before the assessment. This inherently assumes that recent red meat intake is relevant to current EC risk. In cohort studies, baseline assessment of red meat intake is usually assumed to represent a long-term diet and participants were followed-up for 7 to 21 years. Thus, long-term red meat intake was assumed to modulate EC risk. Thus, it is possible that case-control studies and prospective observational studies addressed different questions regarding the red meat intake-EC relationship and thus, reached different conclusions.

Our study has several limitations. First we only investigated a role of red meat intake. Animal derived fat or processed meat has also been reported as risk factors of EC[32-35]. We focused on red meat in the context of consumers' intuition contrasting red meat from white meat or fish as people usually classify red meat as one of representative category when they shop at a market or order at a restaurant. Second we often used rather arbitrary intake as a representative intake of corresponding category when the mean or median intakes were not provided in dose-response meta-analysis. Since the representative dosage should not be missed in each range for dose-response meta-analysis, such an extrapolation can be accepted as technically inevitable.

Nonetheless this study has strength in that it provides updated evidence regarding the relationship between red meat intake and EC risk by incorporating recently published prospective studies.

In summary, our meta-analysis found a significant linear association between red meat intake and EC risk based on case-control studies but this was not confirmed in prospective studies. This discrepancy seems to be attributable to the differences in robustness against biases and reference year of assessment of red meat intake between the retrospective and prospective studies. When the implication of the current study is addressed, however, it should be considered that the quality of evidence from cohort studies be higher because it is more likely to represent the real world situation.

COMMENTS

Background

The incidence of endometrial cancer (EC) is increasing as the life styles become westernized globally. EC is estimated to be the fourth most common cancer in females in the. The association between red meat intake and the risk of EC is currently unclear.

Research frontiers

While past meta-analysis suggested evidence for a significant inverse association and quantified that 100 g/d intake was significantly associated with an approximately 60% increased risk of EC, this meta-analysis included only one prospective study, which could not be sufficient at this time because several prospective studies have been published afterward.

Innovations and breakthroughs

The aim of the current study was to conduct an up-to date dose-response meta-analysis of red meat consumption and the risk of EC based on case-control studies and prospective cohort studies.

Applications

This meta-analysis found a significant linear association between red meat intake and EC risk based on case-control studies but this was not confirmed in prospective studies. More results from prospective studies with long-term follow up are in need to confirm the association between red meat intake and the risk of EC.

Terminology

EC is a carcinoma originated from the inner mucous membrane of mammalian uterus, which is also referred as uterine cancer or corpus cancer.

Peer-review

This review article is well written and will contribute to the clinical practice of the readers.

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