Perna canaliculus (Green-Lipped Mussel): Bioactive Components and Therapeutic Evaluation for Chronic Health Conditions

Samantha Coulson, Talia Palacios and Luis Vitetta

Abstract *Perna canaliculus* (Green-Lippped Mussel) is found only in New Zealand waters and is cultivated and manufactured for both the food and nutraceutical industry world-wide. *P. canaliculus* has traditionally been used as a therapeutic to treat various arthralgias in both humans and animals; however, clinical research reports provide conflicting results. Numerous in vitro studies have reported anti-inflammatory activity of the mussel under various conditions and also demonstrated a synergistic effect with pharmaceutical medications such as non-steroidal anti-inflammatory drugs (NSAIDs) with *P. canaliculus* protecting the gastrointestinal mucosal lining against such medications. It is proposed that the anti-inflammatory activity demonstrated by *P. canaliculus* is predominantly due to the lipid fraction, however, among the major classes of compounds found in mussel meat, proteins and peptides are the largest with isolates demonstrating various anti-microbial, anti-inflammatory, anti-oxidant, bioadhesive and anti-hypertensive activities. A review of the bioactive components, their function and therapeutic application is outlined in this chapter. Furthermore, we hypothesise and provide supportive evidence that the gastrointestinal microbiota play an important role in disease processes such as Rheumatoid arthritis and Osteoarthritis and also in the efficacy of *P. canaliculus* in chronic inflammatory conditions. The metabolic capacity of intestinal microbiota can modify bioactive food components altering the hosts' exposure to these components, potentially enhancing or diminishing their health effects. Understanding the interaction of the bioactive compounds in *P. canaliculus* with commensal and pathogenic bacteria may facilitate the development of novel interventions to control intestinal and extraintestinal inflammation.

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Keywords *Perna canaliculus* · Green-Lipped mussel · Anti-inflammatory · Gastrointestinal tract · Microbiota

Abbreviations

AA	Arachidonic acid
ARA	American rheumatism association
5-HETE	5-Hydroxyeicosatetraenoic acid
CRP	C-reactive protein
DHA	Docosahexaenoic acid
ESR	Erythrocyte sedimentation rate
EPA	Eicosapentaenoic acid
E/LFT	Electrolytes/liver function test
FFAs	Free fatty acids
FBC	Full blood count
GIT	Gastrointestinal tract
GC-MS	Gas chromatography-mass spectrometry
GSRS	Gastrointestinal symptom rating scale
HAQ	Health assessment questionnaire
Hb	Haemoglobin
IBD	Inflammatory bowel disease
IgG	Immunoglobulin G
IL-1	Interleukin-1
KOH	Potassium hydroxide
LCPUFA	Long chain polyunsaturated fatty acids
LOX	Lipoxygenase
NSAIDs	Non-steroidal anti-inflammatory drugs
NMR	Nuclear magnetic resonance
NF-kB	Nuclear factor-kappa B
OA	Osteoarthritis
PCR	Polymerase chain reaction
PGE2	Prostaglandin E2
PMN	Polymorphonuclear leukocytes
PG-PS	Peptidoglycan-polysaccharides
RA	Rheumatoid arthritis
RBC	Red blood count
SCFA	Short chain fatty acids
SFE	Supercritical fluid extraction
SF-12V2™	SF-12 heath questionnaires
TNF-α	Tumour necrosis factor—α
TXB2	Thromboxane-2
WOMAC	Western Ontario McMaster Universities arthritis index
VAS	Visual analogue scale

1 Introduction

Perna canaliculus belongs to the class *Bivalvia*, the phylum *Mollusca* and family *Mytilidae*. The genus *Perna* contains species of both green and brown mussels located predominantly in the Southern Hemisphere but also found in North Africa and the northern coasts of South America, with Paleontological data dating the genus back to the Eocene period (60 million years ago) (Wood et al. 2007). Three well-defined species are recognised in the *Perna* genus that includes *P. viridis* (Asian green mussel) found through Indo-Pacific regions, *P. perna* (brown or rock mussel) found through Atlantic regions and *P. canaliculus* which is endemic to New Zealand waters only and has been commercially and sustainably farmed since the early 1970s (Wood et al. 2007). *P. canaliculus* is distinguished from other mussel species by the bright green stripe around the posterior ventral margin and inside the lip of its shell (see Fig. 1) (Wolyniak et al. 2005). Numerous bioactive compounds have been identified in both the *Mytilus* and *Perna* genera of mussels, but it is *P. canaliculus* that has been most comprehensively studied for medicinal purposes. It has supported the development of commercial therapeutic products to treat arthralgia in humans and animals. It has also been assessed as an adjunct therapy for rheumatoid arthritis (RA), asthma and gastrointestinal tract (GIT) complaints (Gibson et al. 1980; Gibson and Gibson 1998; Coulson et al. 2012; Mickleborough et al. 2013). *P. canaliculus* is manufactured in New Zealand as unadulterated freeze-dried whole (i.e. without shell) extract of the mussel meat; as whole with the lipid fractions removed and as a concentrated lipid extract only.

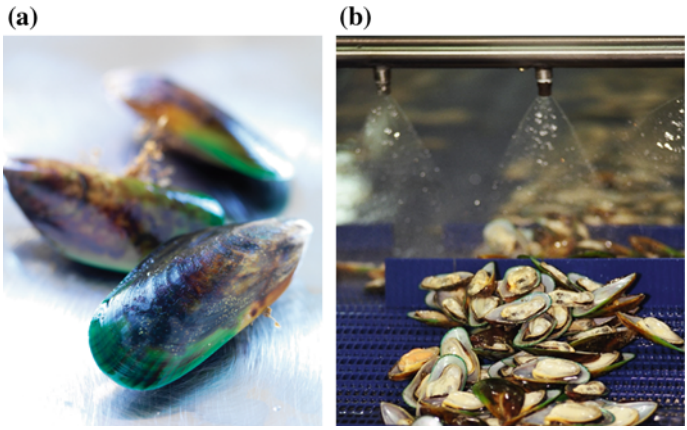

Fig. 1 *Perna canaliculus* **a** mussel shell **b** mussel in shell, note green lip on inside and outer posterior ventral margin (used with permission from Aroma New Zealand)

1.1 *Growing and Harvesting* Perna canaliculus

Interest in the application of *P. canaliculus* for arthritic conditions began in the 1960s, when research was undertaken to discover new natural compounds from marine organisms, which included *P. canaliculus* to treat cancer. *P. canaliculus* extract did not provide significant results for cancer outcome measures, but it was found that the study participants who also suffered from arthritis reported less pain and stiffness and improved mobility when taking the extract (Kendall 2000). It was also observed that coastal Maoris of New Zealand, whose staple diet consisted of *P. canaliculus* had a lower incidence of arthritis than Maoris residing in land (Halpern and Georges 2000; Brien et al. 2008a). Research has therefore focused on the anti-inflammatory capabilities of *P. canaliculus* extract and its fractions. Mussels are farmed in New Zealand using long-line technology around sheltered, in-shore areas such as the Marlborough Sounds. The spat, or seed, is collected from farmers suspending the spat catching lines or by collection of seaweed that spat (<5 mm in size) have naturally adhered to. The collected spats are then resettled onto nursery lines and grown for 3–6 months (10–30 mm) after which the juveniles are resettled onto thicker ropes and grown to maturity (90–120 mm) for another 12–18 months depending on the growing conditions. Once harvested, mussels are quickly transported to the processing plant where they are chilled (<10°C) in holding tanks. Mussels are then placed in a continuous centrifuge that separates the meat from the shell, which is then placed in refrigerated tanks and a natural anti-oxidant is added to improve stability. Mussel meat is then freeze-dried (lyophilised) at −20°C for 20–22 h. The freeze-dried product is then milled into a fine powder (FAO 2014; Aroma 2014). The lipid extract from the stabilised freeze-dried mussel powder is obtained by a supercritical fluid extraction process (SFE) using liquefied carbon dioxide (CO_2).

1.2 *Nutritional Content of* Perna canaliculus

Whole *P. canaliculus* extract consists of a complex mixture of compounds being predominantly 55–60 % protein, 5–15 % carbohydrates, 5–15 % glycosaminoglycans (including chondroitin sulphate and heparin), 3–5 % lipids, 5 % minerals and 0.5–4 % water (Ulbricht et al. 2009). Vitamins A, D3, E and B12 are also present. The concentrated lipid extract contains a complex profile of fatty acids classes including sterol esters of cholesterol and desmosterol/brassicasterol, triglycerides, free fatty acids (FFAs), sterols and phospholipids (Ulbricht et al. 2009; Whitehouse et al. 1997; Murphy et al. 2003). Analytical assessment of aqueous and lipid metabolomes by nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS) can clearly demonstrate constitutive differences in mussel species, for example, between *P. canaliculus* and *Mytilus galloprovincialis* (Australian Blue mussel). There are distinguishing patterns of

amino acids, several metabolites, glucose and lipids between the two species, although some of these differences could in part be due to location rather the species (Rochfort et al. 2013). Heavy metals that accumulate in water such as arsenic, mercury, cadmium and lead are also present in the whole mussel meat due to their filter-feeding behaviour; however, heavy metal limits are rigorously monitored (see Table 1). Furthermore, the growing waters from which the mussels are harvested are monitored weekly for biotoxins. If levels exceed the legislational limit in New Zealand, no harvesting of the mussels can take place.

Table 1 Whole *P. canaliculus* extract: typical nutritional evaluation (*Source* Aroma NZ Ltd and Biolane®)

Components	Results and (reference range)
General	
Crude protein (g/100 g)	56–61 (40–70)
Carbohydrate (g/100 g)	9.6–12 (NLT 5.0)
Lipids (g/100 g)	10–10.8 (6–15)
Omega 3 fatty acids (EPA/DHA per 100 g)	2.8–4.5 (NLT 2.0)
Saturated fat %	3.3
Glycosaminoglycans %	3.0 (NLT 1.0)
Ash (g/100 g)	18–21 (4–25)
Moisture (g/100 g)	0.6–4 (0–5)
Vitamins	
Vitamin A (IU/100 g)	131.5–329
Vitamin D3 (IU/100 g)	272–1640
Vitamin E (IU/100 g)	2.8–10.6
Vitamin B12 (μg/100 g)	116
Minerals	
Copper (mg/kg)	4.5–5.6
Zinc (mg/kg)	57–62
Manganese (mg/kg)	15–24
Boron (mg/kg)	28
Chromium (mg/kg)	1.4
Iron (mg/kg)	380–670
Calcium (g/100 g)	1.3–1.5
Phosphorus (g/100 g)	0.84–1.25
Sodium (g/100 g)	3.6–4.8
Potassium (g/100 g)	1.2
Magnesium (g/kg)	4.9–6.8
Nickel (ppm)	1.3
Selenium (mg/kg)	2.5
Iodine (mg/kg)	15.4
Sulphur (g/100 g)	3.9

(continued)

Table 1 (continued)

Components	Results and (reference range)
Amino acids (mg/g)	
Aspartic acid	42.8–44.0
Glutamic acid	51.8–58.8
Serine	18.9–19.5
Histidine	7.8–8.5
Glycine	40.9–43.8
Threonine	18.9–21.2
Arginine	27.0–35.9
Alanine	18.3–24.5
Valine	14.6–16.3
Methionine	8.5–9.5
Phenylalanine	14.8–16.2
Isoleucine	16.0–17.7
Lysine	29.2–51.3
Leucine	17.9–23.6
Proline	14.5–19.7
L cysteine	6.1–6.6
Tyrosine	13.9–15.4
Tryptophan	4.9–5.2
Heavy metals (mg/kg)	
Lead	0.89 (NMT 2.0)
Cadmium	0.56 (NMT 5.0)
Mercury	0.08 (NMT 1.0)
Total arsenic	11 (NMT 15.0)

NLT Not lower than; *NMT* Not more than; *EPA* Eicosapentaenoic acid; *DHA* docosahexaenoic acid

There are no standardisation procedures in place for *P. canaliculus* raw material suppliers. This may result in potential variability between the nutrient profiles and stability of the raw materials. Temperature and season both affect the nutrient profile of the mussels during harvest. As the key constituent responsible for the observed therapeutic activity is not definitively known, lack of standardisation practises may influence therapeutic efficacy between marketed products (Whitehouse et al. 1997, 1999). Early human clinical studies for both osteoarthritis (OA), RA and animal studies have resulted in variable assessments of the efficacy of *P. canaliculus* whole extract powders, reporting both positive (Gibson et al. 1980; Audeval and Bouchacourt 1986) and negative outcomes (Highton and McArthur 1975; Huskisson et al. 1981; Caughey et al. 1983; Larkin et al. 1985). It was only in 1986 when New Zealand manufacturers began stabilising the whole mussel extracts with 3 % tartaric acid (a metal chelator and anti-oxidant)

immediately after removing the flesh from the shell, preventing auto-oxidation, when the activity of *P. canaliculus* whole extract powders began to demonstrate more potent activity (Whitehouse et al. 1997).

2 Clinical Therapeutic Activity

Current opinion concerning the therapeutic efficacy of *P. canaliculus* whole extract and/or the lipid fraction is that, the existing evidence is rather inconclusive for the treatment of OA symptoms, with overall evidence for RA suggesting inefficacy (Brien et al. 2008b; Cobb and Ernst 2006; Ulbricht et al. 2009). Individual studies to assess *P. canaliculus* (whole and lipid extract) for treating joint symptoms of OA have all reported a clinically relevant reduction in joint pain and stiffness. Clinical studies assessing *P. canaliculus* for OA, RA and asthma are presented in Table 2. Systematic reports of little or no conclusive evidence from the available studies are generally due to poor methodological rigour, variations in product stability and dosing, lack of raw material standardisation and use of inappropriate placebos, such as dried fish powder. The majority of studies assessing *P. canaliculus* for RA were conducted in the mid-1970s and early 1980s; the results of which may have been influenced by the lack of product stability during this period. Assessment of efficacy is also difficult due to the variable prescribed dosing patterns (dose and duration) used for both OA and RA symptoms. Importantly, the use of rescue pain medication, in the form of either acetaminophen (paracetamol) or non-steroidal anti-inflammatory (NSAID) medications were inconsistent and poorly reported in these earlier studies and may have further influenced the interpretation of the results. It is now recognised; however, that *P. canaliculus* may have credible pharmacoactivity as demonstrated in animals and in vitro studies, which requires further rigorous scientific investigations to assess efficacy and optimal dosage in humans (Rainsford and Whitehouse 1980; Brien et al. 2008b).

The lipid fraction is obtained by supercritical fluid extraction (CO_2-SFE) from the stabilised, freeze-dried mussel powder that is then combined with olive oil and vitamin E as an anti-oxidant. By using CO_2 as an extracting medium, high temperatures and solvents are not used for extraction, thus maintaining therapeutic activity of the extract. Processing with high temperature activates degrading enzymes within the mussel, namely phospholipases and lipoxygenases that degrades the lipid components (Grienke et al. 2014; Wakimoto et al. 2011). Further, fractionation and analysis of the active components in the whole lipid fraction is difficult due to their instability and concomitant decomposition during the bioassay process (Wakimoto et al. 2011). It is reported that the stabilised SFE lipid fraction significantly improves asthmatic symptoms (hyperpnea-induced bronchoconstriction and mild to moderate atopic asthma) when compared to placebo in humans (see Table 2) (Mickleborough et al. 2013; Emelyanov et al. 2002). The lipid extract has also been assessed for its anti-inflammatory activity with the assessment of serum inflammatory markers such as TNF-α and IL-1β, but with non-significant results

Table 2 Human clinical studies assessing the therapeutic activity of *P. canaliculus* whole extract and SFE lipid-rich fraction

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
Osteoarthritis							
Coulson et al. (2012) Whole extract powder (3,000 mg/day)	Non-blinded, non-randomised pilot	21 Knee OA 8 M/13 F 61.1 ± 12.2 years	None	8 weeks	NSAIDs/paracetamol (variable doses)	WOMAC Lequesne algofunctional index GSRS SF-12v2^{TM} Analytical blood safety assessment (FBC, E/LFT, CRP, ESR)	The standardised extract significantly improved knee joint pain ($p < 0.001$), stiffness ($p = 0.002$) and mobility ($p < 0.001$) Furthermore, GIT symptoms were also significantly improved ($p = 0.005$) Adverse events included reflux (10 %), abdominal pain (5 %), diarrhoea (5 %) and gout (10%)
Coulson et al. (2013) Whole extract powder (3,000 mg/day)	Non-blinded, randomised, comparator-controlled, parallel-group	40 knee OA 12 M/16 F 58.6 ± 8.9 years	Glucosamine sulphate 3,000 mg/day	12 weeks	NSAIDs/paracetamol (variable doses)	WOMAC Lequesne algofunctional index GSRS SF-12V2TM Analytical blood safety assessment (FBC, E/LFT, CRP, ESR) Faecal bacterial profiles	Both the he standardised *P. canaliculus* extract ($p < 0.001$) and Glucosamine ($p = 0.001$) treated groups significantly improved knee joint pain, stiffness and mobility. The GLM group also demonstrated significant improvement in GIT symptoms ($p = 0.02$). Improvement in the Glucosamine group was borderline ($p = 0.044$) *Clostridia* and *Staphylococcus* species were observed to decrease from baseline to week 12 in both treatment groups Adverse events included gastrointestinal symptoms, infections (URTI, gastroenteritis, cutaneous), headache, migraine, falls and angina

(continued)

Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
Gibson et al. (1980) Whole extract powder (1050 mg/day)	Double blinded, randomised controlled	38 OA 1 M/37 F Mean age 69.6 (active) and 68.6 (placebo) years 28 RA 1 M/27 F Mean age 54.1 (active) and 60.6 (placebo) years	Inactive fish capsules [dose not specified]	3–6 months	NSAIDs (Type/dose not specified)	OA and RA outcomes: • Morning stiffness • VAS • Functional index • Time taken to walk 50 feet • Range of joint movement • Patient and physicians global assessment of improvement Additional RA outcomes: • Articular index of joint tenderness • Grip strength Analytical blood safety assessment (Hb, WBC, ESR, rheumatoid serology, serum biochemistry, urine analysis)	The whole extract improved pain and stiffness in 76 % of RA patients and 45 % OA patients. From 66 patients in the trial, 44 suffered from night pain. This was relieved in 17 patients on active treatment and 2 in the placebo group. There was no significant improvement in range of movement or grip strength Adverse events included exacerbation of joint symptoms (9 %), increased joint stiffness (3 %), epigastric discomfort (2 %), increased flatulence (2 %) and nausea (6 %)

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Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
Audeval and Bouchacourt (1986) Whole extract powder (6 capsules/day) (Dosage not specified)	Double blinded, randomised placebo controlled	53 knee OA 16 M/37 F 66 ± 11 years (placebo) 65 ± 10 years (active)	Placebo (not specified)	6 months	NSAIDs (type/dose not specified)	Functional capacity ARA (stage I–IV) VAS Intensity of pain (I–IV) Amplitude of joint mobility Distance of heel-cheek Utilisation of a walking stick Maximum distance walked Patient and Physicians global assessment of improvement	The whole extract significantly improved functional capacity ($p < 0.001$), pain intensity ($p < 0.01$), patients assessment ($p < 0.05$), physicians assessment ($p < 0.01$) compared to placebo. However, morning stiffness improved significantly in the placebo group compared to the treatment group ($p < 0.01$) No adverse events were reported
Zawadzki et al. (2013) Lipid extract 4 capsules (1,200 mg/day)	Blinded, comparative controlled in stage I, non-blinded in stage II	50 knee and/or hip OA 6 M/44 F 65.6 ± 9.5 years (active) 66.7 ± 8.4 years (fish oil)	Fish oil 1,200 mg/day	3–6 months	Paracetamol (dose not specified)	VAS (100 mm) HAQ Analytical blood assessment (WBC, RBC, FBC, LFT, ESR) Adverse reactions	In stage I, the patients from Group A, treated with the lipid extract, showed a statistically significant reduction of pain, improved levels of mobility and activity and 100 % tolerance with no side effects noted. In comparison, patients from Group B, who were treated with fish oil, did not show a notable reduction in pain, there was no significant improvement of mobility or activity and only 71 % tolerance. In stage II, the patients knew that they had taken the lipid extract and those

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Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
							patients also showed a reduction of pain Adverse events included diarrhoea, stomach ache, increased blood pressure, nausea, constipation, headaches and pain in the kidney and liver areas in the fish oil group only
Gibson and Gibson (1998) Lipid extract (210 mg/day)	Double-blinded randomised for first 3 months; Non-blinded, randomised for last 3 months	30 OA 8 M/22 F Mean age 57.3 (lipid) and 52.8 (powder) years 30 RA 2 M/28 F Mean age 46.8 (lipid) and 47.1 (powder) years	GLM extract powder 1150 mg/day	3–6 months	NSAIDs (type/dose not specified)	Articular index Morning stiffness Grip strength VAS Presence/absence of night pain Patient and physicians global assessments Analytical blood assessment (FBC, ESR, rheumatoid factor)	In RA patients, significant improvements were obtained in articular index ($p < 0.01$), morning stiffness ($p < 0.05$) and functional index ($p < 0.01$) in both the lipid and GLM extract powder groups. Grip strength and the VAS did not improve significantly. Night pain improved In OA patients, significant reductions were obtained in articular index ($p < 0.01$), morning stiffness ($p < 0.01$) with improved function index ($p < 0.01$). The VAS significantly improved in the GLM extract powder group only. Grip strength did not change. Night pain improved No obvious difference was demonstrated between the stabilised mussel powder and the lipid extract

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Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
Lau (2004) Lipid extract 4 caps/day for 2 months then 2 caps/day for 4 months Dosage not specified	Blinded, randomised placebo controlled	80 knee OA	Olive Oil capsules	6 months	Paracetamol 2 g/day plus additional amounts for rescue medication		Significant improvement in pain and global assessment
Cho et al. (2003) Lipid extract (4 caps/day) Dosage not specified	Non-blinded, non-randomised, multi-centre	60 knee and hip OA 2 M/52 F Average age 6.4 years	None	8 weeks	NSAIDs discontinued prior to starting study Use of pain relief medication not specified	VAS Lequesne algofunctional index Patient and physicians global assessment	After 4 weeks, 53 % of patients experienced pain relief and improved joint function; this number increased to 80 % after 8 weeks. The grade of pain and functional impairment were reduced significantly ($p < 0.05$) and patient and physician global assessments improved, 87 and 90 % respectively Adverse events included initial transient worsening of arthritic pain (4 %)
Rheumatoid arthritis							
Huskisson et al. (1981) Whole extract powder (900 mg/day)	Blinded, randomised, placebo, cross-over, controlled	30 RA Gender/age not specified	Dried fish capsules (dose not specified)	8 weeks	Analgesics and NSAIDs (dose and type not specified)	VAS Duration morning stiffness Articular index Proximal interphalangeal joint circumference Analgesic requirements	No significant difference obtained between the whole extract powder and placebo for all outcome measures Adverse events included headache, abdominal pain, diarrheoa and constipation

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Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
Caughey et al. (1983) Whole extract powder (1050 mg/day)	Blinded, randomised, placebo controlled	47 RA 11 M/36 F 29–68 years (median 49.4 years)	Dried fish capsules (dose not specified)	12 weeks	Group I: Naproxen 750 mg/day plus *P. canaliculus* extract from week 1–6 Group II: naproxen 750 mg/day plus placebo from week 1–6 From weeks 7–12, naproxen was replaced by a placebo in each group	VAS Grip strength Ritchie articular index Returned analgesic count Patient weekly record of subjective symptoms (severity day/night pain, severity morning stiffness, analgesic requirements and global assessment) Analytical blood assessments (included ESR)	No significant difference obtained between treatment and placebo for all outcomes measures Following withdrawal of naproxen after week 6, there was a high dropout rate with no significant difference between the active and placebo groups Adverse events included skin rash, diarrhoea and upper gastrointestinal symptoms
Larkin et al. (1985) Whole extract powder 6 caps/day (920 mg/day for 3 months then (1180 mg/day) for a further 3 months	Double-blinded, randomised, placebo controlled	35 RA 54–77 years (median 60 years) (active) 48–70 years (median 60 years) (placebo)	Placebo (not specified)	6 months	NSAIDs and prednisolone (n=1)	Ritchie index Grip strength Morning stiffness VAS (100 mm) Analytical blood assessment (ESR, Hb, platelets, globulins, Immunoglobulin's, rheumatoid factor, serum biochemistry)	No significant difference observed between treatment and placebo group for any of the measured parameters. There were a significant number of patients who reported arthritis symptoms worsening while taking the *P. canaliculus* extract ($p < 0.05$)

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Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
Highton and McArthur (1975) Whole extract powder (Dosage not specified)	Double-blinded, randomised, cross-over, placebo controlled	6 RA 1 M/5 F 34–59 years (median 46.5 years)	Placebo (not specified)	12 weeks	Maintenance gold and low dose steroids continued NSAIDS withdrawn 14 days prior to participating Paracetamol permitted for pain relief	Ritchie articular index Number swollen joints Joint range of movement Joint circumference Joint swelling grip strength Time taken to walk 10 m Analytical blood analysis Patient diary record of morning stiffness duration, pain and number paracetamol tablets required	No significant difference observed between treatment and placebo group for all measured parameters. Paracetamol consumption increased during trial Adverse events included increased severity of symptoms
Gruenwald et al. (2004) Lipid extract combined with Ω-3 fish oil (140 and 1832 mg/day respectively)	Non-blinded, controlled	50 RA 25 M/25 F 29–73 years	None	12 weeks	Existing pharmacotherapy continued but not specified	Morning stiffness Joint pain Joint swelling Joint pain intensity	After 12 weeks supplementation there was a significant reduction in the duration of morning stiffness ($p \leq 0.001$), the number of painful joints ($p = 0.001$) and the number of swollen joints ($p = 0.001$). A highly significant reduction in the number of painful small joints was achieved ($p = 0.002$). Successive reduction in pain intensity was observed over the course of the study

(continued)

Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
							Further, 34 (68 %) of patients required pharmacotherapy prior to and during the course of the trial. One of most important outcomes in this trial is that pharmacotherapy could be reduced for 21 of these patients by the end of the treatment period and eliminated for 13 Adverse events included mild nausea
Anti-inflammatory activity							
Murphy et al. (2006) 2 ml/day of the lipid extract containing 241 mg Ω-3 LCPUFA supplying 97 mg EPA and 72 mg DHA. Preparation combined with olive oil and dl-α-tocopherol	Double-blinded, randomised, parallel intervention study	30 healthy people 14 M/16 F	2 ml/day of fish oil containing 181 mg Ω-3 LCPUFA supplying 87 mg EPA and 50 mg DHA. Preparation combined with olive oil and dl-α-tocopherol	6 weeks	None	Serum inflammatory markers—TXB2, PGE2, IL-1β and TNF-α Levels of fatty acids in neutrophils determined at day 0, 42 and 56	Following supplementation, there were no significant changes in inflammatory markers in either of the marine oil-fed groups or between the groups of apparently healthy volunteers. There was a very wide spread of values for most analytes which may have obscured changes with treatment or between treatments

(continued)

Table 2 (continued)

Author GLM/dose per day	Design	Cohort Age (mean ± SD)	Comparator/placebo	Duration	Use of analgesic medication	Outcome measures	Clinical trial results
Anti-asthmatic							
Mickleborough et al. (2013) 8 caps/day (1,200 mg/day) of the lipid extract containing ~72 mg EPA and 48 mg DHA	Double-blind, placebo controlled randomised crossover study	20 asthmatic people with hyperpnea-induced bronchoconstriction (HIB) 12 M/8 F	Olive oil (1,200 mg/day)	8 weeks	Inhaled short acting β2-agonists	Eucapnic voluntary hyperventilation (EVH) challenge (surrogate for an exercise challenge test) Fraction of exhaled nitric oxide (F_ENO) Pulmonary function tests Eucapnic voluntary hyperventilation (EVH) Symptoms of rescue β-agonist use and peak flow measurements Exhaled breath condensate (EBC)	The lipid extract significantly reduced pre- and post-EVH, asthma symptom scores and EBC rescue medication when compared to the placebo group
Emelyanov et al. (2002) 4 caps/day (600 mg/day) of the lipid extract	Double-blind, parallel group, randomised placebo-controlled study	46 people with mild to moderate atopic asthma	Olive oil (600 mg/day)	8 weeks	Inhaled short-acting β2-agonists	Peak expiratory flow rate (PEF) Hydrogen peroxide (H_2O_2) in expired breath condensate (marker of airway inflammation)	There was a significant decrease in daytime wheeze, the concentration of exhaled H_2O_2 and an increase in morning PEF in the lipid extract group compared to the placebo group

WOMAC Western Ontario McMaster Universities Arthritis Index; *GSRS* Gastrointestinal symptom rating scale; *SF-12V2*^{TM} SF-12 heath questionnaires; *FBC* Full blood count; *E/LFT* Electrolytes and liver function test; *RBC* Red blood count; *CRP* C-reactive protein; *ESR* Erythrocyte sedimentation rate; *VAS* Visual analogue scale; *Hb* Haemoglobin; *ARA* American Rheumatism Association; *HAQ* Health assessment questionnaire; *LCPUFA* Long chain polyunsaturated fatty acids; *EPA* Eicosapentaenoic acid; *DHA* Docosahexaenoic acid; *TXB2* Thromboxane-2; *PGE2* Prostaglandin E2; *IL-1* Interleukin-1; *TNF-α* tumour necrosis factor—α; *VAS* Visual analogue scale

(Murphy et al. 2006). The cohort recruited, however, were healthy individuals that did not demonstrate any signs of inflammation, with serum cytokine markers within normal reference ranges before intervention. Such a design is unlikely to answer the question of whether the lipid extract reduces markers of inflammation.

2.1 Dosing

The optimal therapeutic dose of either the whole or lipid extract has not been clearly ascertained and can only be estimated from previous clinical research. It is clear that carefully designed Phase I dose-ranging studies are required to ascertain what the effective prescribed dose should be for both extracts and in various disease contexts. Clinical studies have used a dose range between 1,050 and 3,000 mg/day for the whole extract and 210–1,200 mg/day for the lipid extract in OA patients with dose duration between 8 and 24 weeks. A dose between 900 and 1,180 mg/day of the whole extract and 140 mg/day of the lipid extract has been assessed in RA patients with a dose duration between 8 and 24 weeks and a dose between 300 and 1,200 mg/day of the lipid extract has been assessed for asthma patients with a dose duration of 8 weeks (see Table 2). The recommended dose from the manufacturer for the *P. canaliculus* whole extract is typically 1,500 mg/day and 200 mg/day for the lipid extract. It is unclear, however, if these are the effective therapeutic doses for each extract based on the presented research dose variations.

2.2 Adverse Effects and Toxicity

Adverse events reported in clinical studies assessing both the whole and lipid extract have included mild gastrointestinal events such as reflux, flatulence, epigastric discomfort, fluid retention, nausea and altered bowel habits, headaches and a transient increase in knee symptoms. Apart from these minor events, the intake of *P. canaliculus* is not associated with any serious adverse events and is generally well tolerated. The use of *P. canaliculus* should, however, be avoided by people with allergies to shellfish. Heavy metal poisoning is unlikely to occur (Ulbricht et al. 2009); however, biotoxins may be found in shellfish due to their filter-feeding behaviour and ingestion of large amounts of algae. The majority of the mussel's diet consists of nutrient-rich eukaryotic microalgae, typically diatoms and dinoflagellates, but it is mainly when they ingest harmful algal blooms from the surrounding water that toxins become a serious threat to both the health of the consumer and the mussel industry (Grienke et al. 2014). In New Zealand, the growing waters from which the mussels are harvested are tested weekly for biotoxins and if the levels exceed the National limit harvesting is prohibited. The very strict guidelines now in place ensure that there is very low risk of the mussel products containing any biotoxins. To date, there are no reports of either GLM

extract or the lipid extract interacting adversely with pharmaceutical or nutraceutical medications, but rather they may enhance their therapeutic effect (Rainsford and Whitehouse 1980; Whitehouse and Butters 2003).

2.3 *Gastroprotective Activity*

The SFE lipid-rich fraction has exhibited synergistic anti-inflammatory therapy when combined orally with NSAIDs and analgesic pharmaceutical medications such as prednisone, pentoxifylline or meloxicam in rat models. When administered as tandem therapies to reduce paw swelling in adjuvant-induced arthritis and zymosan-induced paw inflammation in rats, the result was more effective than using therapy alone (Whitehouse 2004; Whitehouse and Butters 2003). The whole stabilised extract powder also demonstrates equally synergistic anti-inflammatory activity when combined with prednisone or meloxicam. Both extracts have NSAID and steroid-sparing effects when administered concomitantly, reducing the effective dose required for the drug and also protecting the gastrointestinal tract (GIT) from the adverse effect of such medications. The stabilised whole extract powder reduced the occurrence of gut lesions in rats, more than the lipid fraction, when combined with NSAIDs in an animal model (Whitehouse and Butters 2003). Lipid and whole extracts reinforce the anti-inflammatory therapeutic activity of acetylsalicylic acid and indomethacin while concomitantly exhibiting gastroprotective activity (Rainsford and Whitehouse 1980). Supportive data indicates that supplementation with whole *P. canaliculus* extract may support GIT function and even show gastroprotective activity when administered with anti-inflammatory and analgesic medications in patients with OA (Coulson et al. 2012, 2013). Further, preliminary evidence has demonstrated that the SFE lipid-rich fraction significantly reduced colonic damage in an inflammatory bowel disease (IBD) animal model (Tenikoff et al.2005) and partially improved selected indicators of intestinal inflammation and intestinal morphology in an animal model of chemotherapy-induced mucositis.

3 Bioactive Metabolites of *Perna canaliculus*

Studies to identify bioactive metabolites within *P. canaliculus* products has led to the evaluation of extracts, hydrolysates and purified components from the fractionated lipids, carbohydrates and proteins present in the mussel meat. A review of these fractions is discussed in depth by Grienke 2014 (Grienke et al. 2014). The fraction(s) responsible for the therapeutic efficacy demonstrated in the OA disease model, both human and animal, is not yet fully defined. Previous claims suggest that the lipid fraction represents the dominant anti-inflammatory component of *P. canaliculus* (Ulbricht et al. 2009; Halliday 2008). Early clinical trials reported mixed results with whole extract powder. Stabilisation of the mussel meat with 3 %

tartaric acid in the 1980s resulted in a more active product (Whitehouse et al. 1997). Furthermore, while Gibson and Gibson (1998) comparing the lipid extract to the whole stabilised powder extract in treating joint symptoms of both RA and OA, both mussel preparations demonstrated significant therapeutic activity with no substantial difference found between either treatment (Gibson and Gibson 1998). The major classes of compounds found in mussel meat (peptides, carbohydrates and lipids) have demonstrated various anti-microbial, anti-inflammatory, anti-oxidant, bioadhesive and anti-hypertensive activities (Grienke et al. 2014).

The content of bioactive metabolites in mussel meat is influenced by the season, life cycle, diet and habitat in which the mussels are grown and can therefore vary between harvests (Fearman et al. 2009; Narvaez et al. 2008). Furthermore, there are evident metabolic differences between mussel species and also within the same species when collected from different locations. For example, metabolomic assessment of the Australian Blue mussels (*Mytilus galloprovincialis*) and *P. canaliculus* found taurine, glycine, lactate, succinate, homarine, ATP, ADP, valine and leucine were elevated in *P. canaliculus* while betaine, isoleucine, acetoacetate and glucose were elevated in *M. galloprovincialis*. Also, analysis of lipid methyl ester derivatives indicted a clear separation between the species, with significantly higher levels of palmitic acid methyl ester (C16:0), cis-5,8,11,14,17 eicosapentaenoic acid methyl ester (C20:5n3) and palmitoleic acid methyl esters (C16:1) obtained from *P. canaliculus,* which overall contained a higher lipid level. These differences are likely due in part to the different environments that each species are grown in lower water temperatures correlating with higher degrees of unsaturated lipids (Rochfort et al. 2013). Experimental studies of bioactive carbohydrate compounds from *P. canaliculus*, is limited, with one report of a glycogen isolate demonstrating anti-inflammatory activity (after *i.v.* administration) against carrageenan-induced arthritis in the footpad of rats (Miller et al. 1993). The authors, however, confirmed the anti-inflammatory activity was lost when the glycogen isolate was treated with either potassium hydroxide (KOH) or proteinase-K, proposing that the anti-inflammatory activity was actually due to the protein moieties associated with the glycogen macromolecule.

3.1 Bioactive Proteins, Peptides and Amino Acids

Approximately 70 % of whole mussel meat is protein. The anti-inflammatory and immunomodulating activity of the fractionated extracts of whole extract powder in animal and in vitro models have suggested that the predominant active agent is associated with a protein moiety or is itself a protein macromolecule; however, supportive research for a bioactive high molecular weight protein is currently limited (Couch et al. 1982; Miller et al. 1993; Mani and Lawson 2006; Grienke et al. 2014). Current research has reported anti-bacterial, anti-fungal, anti-inflammatory, anti-hypertensive, anti-oxidant, anti-thrombin and anti-coagulant bioactive proteins, peptides and amino acids from various mussel species.

The only bioactive protein identified from *P. canaliculus* is pernin from the cell-free haemolymph. It is an aggregating, non-pigmented, glycosylated protein extract composed of 497 amino acids with a particularly high content of histidine and aspartic acid residues. Pernin can act as a serine protease inhibitor but only demonstrates weak anti-thrombin activity. The pernin content from homogenise whole mussel meat averages 0.2 mg per mussel (Scotti et al. 2001).

Anti-microbial peptides (AMPs) are also present in the mussel haemolymph and are a vital part of the mussel's innate immunodefense system, protecting it from bacterial, fungal and viral attack. AMPs have been a focus in marine mussel research, particularly in the Blue mussel (*Mytilus edulis)* and the Mediterranean (or Blue) mussel (*Mytilis galloprovincialis)* species. Several cysteine-rich peptides from *M. edulis* were reported to be potent bactericides (i.e. against both Gram-positive organisms, e.g. *Enterococcus faecalis, Staphylococcus aureus* and Gram-negative bacteria, e.g. *Escherichia coli* bacteria) and anti-fungal (i.e. *Neurospora crassa* and *Fusarium culmorum*) (Charlet et al. 1996). The AMPs were identified as isoforms from the peptide families of defensins, mytimycin and mytilins with big-defensins (and mytimacins) also being described (Charlet et al. 1996; Grienke et al. 2014). Crustacean haemolymph, particularly from the crab, contains a multitude of AMPs that participate not only as endogenous antibiotics but may also have a role in inflammation, wound repair and regulation of the adaptive immune system. This has generated some interest in using marine peptides for pharmaceutical developments (Fredrick and Ravichandran 2012). Furthermore, fermented *M. edulis* is reported to contain peptides that inhibit angiotensin I converting enzyme (ACE) with the anti-hypertensive activity confirmed in vivo rat models (Je et al. 2005). An anti-coagulant peptide has also been identified in *M. edulis* (Jung and Kim 2009). Anti-inflammatory activity of proteinaceous fractions in carrageenan-induced footpad swelling in rats was expressed only when following *i. p.* or *i.v*. injections (see Table 3). One study (Miller and Ormrod 1980) compared *i. p.* to orally administered whole extract powder which may or may not have been a stabilised extract. *P. canaliculus* extract powder may also inhibit the production of prostaglandins in rats (Miller and Wu 1984). The therapeutic activity of mussel protein and peptide fractions has not yet been investigated in humans.

3.2 Bioactive Lipid Fractions

The concentrated lipid extract of *P. canaliculus* contains a complex profile of five main lipid classes that include sterol esters (cholesterol and desmosterol/brassicasterol), triglycerides, free fatty acids (FFAs), sterols and phospholipids (Ulbricht et al. 2009; Whitehouse et al. 1997; Murphy et al. 2003). The fatty acid and sterol composition of the mussel lipid is influenced by water temperatures in which the mussel is grown and also the mussels' diet which includes marine phytoplankton, dinoflagellates and zooplankton (Rochfort et al. 2013; Murphy et al. 2003). Approximately 90 fatty acids are present in the

Table 3 Pharmacological activity of protein fractions from *P. canaliculus* in animal and in vitro models

Author	Design	Fractions	Comparator	Method of delivery	Outcome measures	Results	Duration
Miller and Ormrod (1980)	Male and female dark Agouti rats injected with 0.1 ml of a 2 % carrageenan solution into footpad to induce oedema	Freeze dried powder ground (ORA-C) and suspended in saline at parenteral doses ranging from 100 to 1500 mg/kg	Control group with saline injected into footpad Comparator anti-inflammatory used: aspirin (200 mg/kg), phenylbutazone (200 mg/kg) and indomethacin (5 mg/kg) dosed orally	Administered either IP using 18G needle or by gastric lavage	Footpad thickness measured with an engineer's micrometer gauge. Detectable swelling was demonstrated 2 h after challenge and peaked at 4 h Measurement of footpad thickness made before the injection and 2, 4 and 6 h later	Higher doses of ORA-C (1500–500 mg/kg) administered IP produced significant reductions in foot pad swelling compared to untreated animals. Lower doses of ORA-C (200 mg/kg) showed significant anti-inflammatory activity but only if pre-treatment at the same dose had been carried out for several days prior to the carrageenan challenge i.e. a cumulative effect No significant anti-inflammatory activity was seen when ORA-C was administered orally	Foot pad swelling measured before carrageenan challenge and 2, 4 and 6 h after

(continued)

Table 3 (continued)

Author	Design	Fractions	Comparator	Method of delivery	Outcome measures	Results	Duration
Couch et al. (1982)	Female dark Agouti rats injected with 0.1 ml of a 2 % carrageenan solution into footpad to induce foot oedema	1. ORA-C—unfractionated mussel prep dosed at 300 mg/kg 2. ORA-F1—granular, water insoluble material removed from ORA-C and dosed at 150–500 mg/kg (a) ORA-F1—denatured by autoclaving (b) ORA-F1—proteolysed (NH3HCO3/trypsin) (c) ORA-F1—proteolysed (NH3HCO3/no trypsin)-control (d) ORA-F2 - ORA-F1 dialysed, freeze-dried at 185–500 mg/kg	Control group with saline injected into footpad	ORA-C (300 mg/kg), ORA-F1 (150–500 mg/kg), ORA-F2 (500 mg/kg), saline (control) administered IP injection Foot pad measurements 4 h after challenge	Hind footpad thickness measured 2 and 4 h after challenge	ORA-C, ORA-F1, ORA-F2: significantly reduced foot pad swelling Autoclaving and trypsin treated ORA-F1 destroyed most of the anti-inflammatory activity	IP injection 48, 24 and 2 h before carrageenan challenge, foot pad swelling measured 2 and 4 h after challenge

(continued)

Table 3 (continued)

Author	Design	Fractions	Comparator	Method of delivery	Outcome measures	Results	Duration
Miller and Wu (1984)	Male and female dark Agouti rats grouped for breeding	Whole extract powder dosed orally in chow at a ratio of 12.5 g extract to 1 kg of chow	Normal chow diet group	Oral	Conception, Parturition Foetal development	Parturition and foetal development was delayed in animals fed the whole extract diet. There was no significant difference in offspring weights. These results indicate whole extract powder demonstrates an NSAID-like effect inhibiting prostaglandin biosynthesis (prostaglandin inhibitors interfere with ovulation and prolong gestation period in the rat)	30 days
Miller et al. (1993)	Female dark Agouti rats with carrageenan-induced footpad oedema	Phenol—extracted mussel glycogen (0–25 mg) Protein degraded glycogen extract (10 mg) Lipid degraded glyco gen extract (10 mg)	Untreated control group	Intravenous injection	Foot pad oedema	Glycogen extract significantly reduced foot pad swelling but activity was lost when protein or lipid fractions were removed	Not indicated

(continued)

Table 3 (continued)

Author	Design	Fractions	Comparator	Method of delivery	Outcome measures	Results	Duration
Mani and Lawson (2006)	In vitro Cytokine and IgG producing cell lines (V2E9, THP-1, L-929, U-937, A375. S2, Jurkat E6-1, EL-4, CTLL-2, LS174T, and 7TD1)	1. Freeze-dried whole powder—HCL treated (0, 5, 15, 20 and 25 µg) 2. Freeze-dried whole powder—tween 20 treated (0, 5, 15, 20 and 25 µg) 3. Proteinase-K treated tween 20 extracts 4. Glycogen extract of whole powder	Untreated control	In vitro	Secretion of IgG, TNF-α, IL-1, IL-2, IL-6 and colorimetric ovine cyclooxygenase (COX) assay	Both HCL and tween 20 significantly reduced IgG expression. Tween 20 extract significantly reduced cytokine expression and COX-1 and COX-2 activity. Activity was lost when treated with proteinase. Glycogen rich extract decreased COX-1 and COX-2 activity but less so than tween 20 extract. Activity was lost when it was treated with proteinase	

IP Intraperitoneal; *ORA-C* Oedema reducing agent-crude; *ORA-F* Oedema reducing agent-fraction; *HCL* Hydrochloric acid; *IgG* Immunoglobulin G; *COX* Cyclooxygenase

concentrated lipid extract with the omega-3 (Ω-3) FFAs making up approximately 40 % of the fatty acid content in the lipid extract with docosahaexanoic acid (DHA) and eicosapentaenoic acid (EPA) accounting for 84 % of the Ω-3 PUFA (Murphy et al. 2003; Wolyniak et al. 2005; Lee et al. 2009). The FFA fractions and to a lesser extent the triglyceride fractions, are reported to be the only lipids inhibiting cyclooxygenase (COX) isoforms (McPhee et al. 2007; Wakimoto et al. 2011; Whitehouse et al. 1997; Macrides 1997). Furan fatty acids (F-acids) have been identified as minor components of the fatty acids in the lipid extract and have shown anti-inflammatory activity in rat models of adjuvant-induced arthritis, more than that of EPA (Wakimoto et al. 2011). Further, another single phospholipid compound was isolated from freeze-dried whole powder and identified as lyso-lecithin which demonstrated anti-histamine activity in an ex vivo experiment (Kosuge et al. 1986). The investigation of single lipid components is extremely difficult due to the instability of the extract during the purification process; analytical studies have tendered to focus in characterising lipid extracts rather than identifying single lipid compounds (Grienke et al. 2014).

3.2.1 Anti-inflammatory Activity

The anti-inflammatory activity of the CO_2-SFE lipid-rich fraction isolated from stabilised freeze-dried mussel meat has been demonstrated using in vitro analysis, particularly decreasing leukotriene (LTB4 and 5-HETE) and COX metabolite (COX-1 and COX-2) synthesis using variable doses of the SFE lipid-rich extract or further fractionations from this preparation dosed orally (Whitehouse et al. 1997; Dugas 2000; McPhee et al. 2007; Treschow et al. 2007; Macrides 1997). Adjuvant-induced arthritis rat studies have further analysed the anti-inflammatory and pain reducing effects of whole CO_2-SFE lipid-rich fractions and some fractions administered orally, demonstrating the SFE lipid-rich fractions reduce paw swelling and pain when compared to control groups being equal or superior to oral NSAIDs such as naproxen (see Table 4) (Whitehouse et al. 1997; Lee et al. 2009; Whitehouse 2004; Wakimoto et al. 2011; Butters and Whitehouse 2003). Further, it is reported that a fractionated FFA class exhibited greater anti-inflammatory activity at a lower dosage (30 mg/kg) and for a shorter duration (5 days) when compared to the significant anti-inflammatory activity demonstrated for the crude lipid component, a SFE lipid-rich fraction (100 mg/kg) administered for 15 days to adjuvant-induced arthritis rats via subcutaneous injection (Singh et al. 2008).

A further hypothesis for the anti-inflammatory activity demonstrated in in vitro and animal models, beyond that of reducing inflammatory cytokines (LTB4, 5-HETE, COX-1), is that the lipid-rich fraction may beneficially influence change in protein expression related to arthritis (Lee et al. 2008). Lee et al. (2008) conducted a proteomic study examining the effect that the lipid-rich fraction had on protein expression in splenocytes from adjuvant-induced arthritis rats. They found that in rats administered the lipid-rich fraction, six particular proteins (related to metabolism) were decreased while malate dehydrogenase (MDH), which is

Table 4 Therapeutic activity of SFE lipid-rich fractions from *P. canaliculus* in animal and in vitro analysis

Author	Design	Fractions	Comparator	Method of assessment	Outcome measures
Experimental arthritis					
Whitehouse et al. (1997)	Wistar and dark Agouti rats with either (a) adjuvant-induced polyarthritis or (b) collagen(II)-induced auto-allergic arthritis	An SFE lipid-rich extract from stabilised mussel powder 15 mg/kg Oral administered to rats with (a) aqueous dispersions prepared with 0.2 % Tween-20 as a suspending agent or (b) un-emulsified lipids diluted into olive oil	Naproxen Ibuprofen Dried mussel powder (300 mg/kg) Plant oils	Rear paw inflammation was measured with a micrometer. Forepaw inflammation was assessed arbitrarily on a scale of 0-4+ An independent observer assigned an overall arthritis score to all animals based on paw/tail inflammation and overall condition/mobility	Dried mussel powder and the SFE lipid extract both substantially reduced rear and front paw swelling when compared to no treatment and naproxen
Rainsford and Whitehouse (1980)	NSAID-induced gastric damage Wistar rats				
Butters and Whitehouse (2003)	Adjuvant-induced polyarthritis in rats	Various GLM extract preparations including both stabilised and un-stabilised whole mussel powder (300 mg/kg) and SFE lipid-rich extract 20 mg/kg) dosed orally	NSAIDs	Rear and front paw swelling after 4 days of supplementation	The SFE lipid extract substantially reduced paw swelling with the whole extracts demonstrating mixed results with the stabilised whole powder showing greatest paw swelling inhibition
Whitehouse and Butters (2003)	Collagen-induced polyarthritis (relevant to RA) in Wistar rats Persistent inflammation engendered with insoluble calcium salts	SFE lipid-rich extracts from stabilised mussel powder (20 mg/kg) and stabilised whole mussel powder (300 mg/kg) dosed orally	NSAIDs Prednisone	Rear and front paw swelling	Both the SFE lipid extract and stabilised whole mussel extract substantially reduced paw swelling when used alone or when combined with steroid or NSAID medications. Less gastric injury were observed in

(continued)

Table 4 (continued)

Author	Design	Fractions	Comparator	Method of assessment	Outcome measures
	in Wistar rats relevant to OA in rats				the rats treated with the whole mussel extract
Whitehouse (2004)	Rats with pre-established adjuvant arthritis Rats inflamed with zymosan (chronic irritant and, activator of complement and inducer of fibrosis)	SFE lipid rich extract from stabilised mussel powder (20 mg/kg) dosed orally	Pentoxifylline (PTX) Prednisone	Rear and front paw swelling	When prednisone, PTX and SFE lipid extract were each given alone, they were not able to reduce established arthritis or prevent fibrosis and the co-administration of prednisone and PTX has limited effect. But co-administration of the SFE lipid extract amplified the anti-inflammatory potency of either prednisone or PTX The SFE lipid extract would seem a suitable PTX synergistic agent for anti-TNF therapy
Lee et al. (2009)	Adjuvant-induced arthritis using *Mycobacterium butyricum* in male Sprague-Dawley rats	SFE lipid-rich extract dosed orally at 25 mg/kg	Olive oil (300 µl) and Naproxen (20 mg/kg) were fed as vehicle and positive control	Measurement of pain scores: The number of pain-related responses, represented by vocalizations, was recorded during 10 flexions of the tarsotibial joints of the adjuvant-injected paw. Results were expressed as the mean number of vocalizations	The SFE lipid-rich extract was able to control pain at the initial phase of its administration; with similar efficacy to that observed with Naproxen. The pain scores slowly increased again in the group of rats treated with the SFE extract after day 9–14. The Naproxen-treated rats remained pain-free while treated. Both Naproxen and the SFE extract decreased the levels of the

(continued)

Table 4 (continued)

Author	Design	Fractions	Comparator	Method of assessment	Outcome measures
					pro-inflammatory cytokines TNF-α and IFN-g, and increased that of IL-10. Administering olive oil had no effect on cytokine secretion. Rats treated with the SFE extract were apparently cured after 1 year
Wakimoto et al. (2011)	Adjuvant-induced arthritis using *Mycobacterium butyricum* in female Wistar rats	Isolated furan fatty acids from the SFE lipid-rich extract 1–10 mg/kg administered orally	Control Naproxen (10 mg/kg) SFE lipid-rich extract (200 mg/kg)	Paw swelling	Furan fatty acids (minor component of the fatty acids in the lipid extract) exhibited more potent anti-inflammatory activity than EPA but the whole SFE lipid-rich extract demonstrated the most potent AI activity compared to the furan fatty acids
Singh et al. (2008)	Adjuvant-induced arthritis using *Mycobacterium* tuberculosis in male long Evans rats	SFE lipid-rich fraction from stabilised freeze-dried mussel powder s.c. injection dosed at 50 and 100 mg/kg (crude lipid) via s.c. injection Further fractionation of a novel FFA class (C18:4, C19:4 and C21:5) s.c. injection at a dose of 30 mg/kg	Olive oil control Piroxicam (2 mg/kg)	Rear paw swelling Daily body weight changes Subjective disease activity in both the fore and rear paws	Compared to the olive oil control groups, the crude lipid group (50 and 100 mg/kg) did not alter paw swelling or body condition after 5 days, but at 15 days administration, the 100 mg/kg group did respond significantly both in reduced paw swelling and improved body condition

(continued)

Table 4 (continued)

Author	Design	Fractions	Comparator	Method of assessment	Outcome measures
					In contrast, the FFA class group (30 mg/kg) significantly reduced paw swelling and deterioration of body condition at 5 days administration and was equipotent to piroxicam (2 mg/kg/day) Overall, the FFA class exhibited greater AI activity at a lower dose (30 mg/kg) and for a shorter dosage period (5 days) when compared to the significant AI activity obtained for the crude lipid component (100 mg/kg/15 days)
In vitro studies					
McPhee et al. (2007)	In vitro	• SFE lipid-rich extract • Purified PUFA (saponified by KOH hydrolysis) from the lipid extract • Protease lipid extract from whole mussel meat • Protease-lipase lipid extract Various doses used	Fish oil *M. edulis* lipid extract Purified PUFA (saponified by KOH hydrolysis) Indomethacin	COX inhibition assay and COX metabolite analysis	The SFE lipid-rich extract exhibited strong concentration dependent inhibition of both COX-1 and COX-2 and was more inhibitory than indomethacin at comparable concentrations Hydrolysed lipid extract demonstrated 10 times more inhibitory activity against COX than non-hydrolysed lipid extract

(continued)

Table 4 (continued)

Author	Design	Fractions	Comparator	Method of assessment	Outcome measures
					Hydrolysed fish oil exhibited similar activity to that of the hydrolysed mussel lipid extract The SFE free fatty acid fraction, and to a lesser extent the SFE triglyceride fraction, were the only lipid classes to exhibit strong inhibition of the COX isoforms The *P. canaliculus* and *M. edulis* total lipid extracts moderately inhibited COX-1 and COX-2. Inhibition was increased after the mussel extract were saponified by KOH hydrolysis, indicating that the FFA fraction is likely to be in part responsible for the anti-COX activity
Treschow et al. (2007)	In vitro	FFA class isolated and purified from the SFE extract and then rigorously fractionated to isolate bioactive constituents		LOX inhibition assay involving human neutrophils challenged with AA and assayed for LTB4 and 5-HETE	The FFA fraction that demonstrated high bioactivity in inhibiting LTB4 and 5-HETE synthesis was identified as DHA. Other novel compounds with AI activity were identified as C18:4, C19:4, C20:4 and C21:5

(continued)

Table 4 (continued)

Author	Design	Fractions	Comparator	Method of assessment	Outcome measures
Macrides et al. (1997)	In vitro	Ω3 Tetraenoic fatty acids: C18:4, C19:4 and C20:4 PUFAs	None	Inhibition of Leukotriene ((LTB4) produced from stimulated human neutrophils was used as an in vitro screening method to test the efficacy of purified PUFAs for AI activity	The two most active fractions obtained from the separations inhibited LTB4 formation by 64 and 47 % respectively (at 1:100 dilution)
Whitehouse et al. (1997)	In vitro	Fatty acid methyl esters prepared from SFE lipid extract subfractions at various concentrations	None	Leukotriene biosynthesis by human PMNs. Inhibition of arachidonate induced leukotriene-B4 and 5-HETE synthesis in A-23187-activated human PMN	Four of the fractions were effective in inhibiting 5-LOX and 5-HETE with the active fractions containing polyunsaturated acids with 4, 5 or 6 double bonds The unfractionated SFE material also inhibited prostaglandin E2 production from endogenous arachidonate by stimulated human blood monocytes The SFE lipid extract is a potent but slow acting anti-inflammatory agent
Dugas (2000)	In vitro	An SFE lipid-rich extract from stabilised mussel powder Dose NS	None	Leukotriene biosysnthesis by human monocytes (normal and allergic donors). Production of LTB4 by normal and allergic human monocytes when challenged with IL-4	The SFE lipid extract inhibited 5-LOX pathway in both normal and allergic human monocytes when challenged with IL-4

(continued)

Table 4 (continued)

Author	Design	Fractions	Comparator	Method of assessment	Outcome measures
Gastrointestinal protection					
Tenikoff et al. (2005)	Dextran (2 %) sulfate sodium—induced IBD in male C57BL/6 mice	SFE lipid rich-fraction from stabilised freeze-dried mussel powder dosed orally (5 mg lipids/day)	Olive oil Fish oil (55 mf EPA/DHA/day)	Myeloperoxidase (MPO) activity = a measure of neutrophil infiltration Body weight Rectal bleeding Stool consistency Overall condition (disease activity index—DAI) Colon damage—histopathology	SFE lipid-rich fraction significantly reduced body weight loss, DAI scores, crypt area losses and cecum and colon weights compared to the fish oil group. MPO activity was not significantly affected by any treatment
Torres et al. (2008)	Intestinal mucositis model in female dark Agouti rats injected with 5-fluorouracil (5-FU) (150 mg/kg i.p.)	High dose lipid extract—800 µl/day Low dose lipid extract—400 µl/day	Fish oil Olive oil Saline	Small intestinal weight Food intake MPO activity Histological damage Sucrose breath test (small intestinal sucrase activity and absorptive function) Biochemical sucrase activity (biomarker of total small intestinal sucrase activity and absorptive function)	SFE lipid-rich fraction treatment in rats with 5-FU-induced mucositis only minimally decreased indicators of intestinal integrity producing lower histological damage and high dose Lyprinol group had longer crypts and increased proliferation in the mid small intestine

AI Anti-inflammatory activity; *PMN* Polymorphonuclear leukocytes; *LOX* Lipoxygenase; *5-HETE* 5-Hydroxyeicosatetraenoic acid; *KOH* Potassium hydroxide; *AA* Arachidonic acid; *s.c.* Subcutaneous

specifically related to glucose, metabolism was increased, possibly equating to a decline in glucose levels available for the activation of major histocompatibility complex class I (MHC-I). MHC-1 gene expression contributes to autoimmune diseases. Elevated levels of MDH can possibly decrease free glucose in the cytoplasm by converting pyruvate into malate (Lee et al. 2008).

Rat studies reporting anti-inflammatory activity with the SFE lipid-rich fraction have typically orally administered 20 mg/kg of body weight per day (highest 100 mg/kg/day) to rats with adjuvant-induced arthritis. The current dosage recommendation for humans, however, is 100–200 mg per day of the SFE lipid-rich fraction (administered with olive oil as a carrier). When comparing the dosages, using a 70 kg person for example, the doses used in rat studies are typically 7–14 times higher than that which is recommended for relief from arthritis-induced inflammation and pain in humans. How the low dose for human consumption was recommended, when rat studies demonstrated anti-inflammatory and pain relief at high doses, is not clear. It may be due to variations of metabolic capacity between rats and humans.

3.2.2 Anti-asthmatic Activity

The inhibition of leukotriene and prostaglandin E series production by the lipid-rich fractions has led to its assessment in both animal and human asthma models. Significant reductions in the development of allergic inflammation and airway hyperresponsiveness (rat model) (Wood et al. 2010) and asthma symptoms were attained in humans (Mickleborough et al. 2013; Emelyanov et al. 2002). Mickleborough et al. (2013) conducted a placebo controlled, double-blind randomised crossover study in patients with mild to moderate asthma (n = 20) who were given either eight capsules per day of a stabilised SFE lipid-rich extract (providing 72 mg EPA and 48 mg DHA) or placebo (olive oil) for 3 weeks duration, then followed by a 2 week 'washout' period before treatments were crossed over. The study showed that the lipid-rich fraction significantly reduced airway inflammation and the bronchoconstrictor response to dry air hyperpnea. The lipid-extract group also benefited by reduced asthma symptom scores and their lesser use of rescue medication compared to the placebo group (Mickleborough et al. 2013). Emelyanov et al. (2002) also demonstrated in a double-blind, parallel group, randomised, placebo-controlled study (n = 46) that the stabilised SFE lipid-rich fraction when supplemented at four capsules/day for 8 weeks duration, significantly decreased day time wheeze, concentration of exhaled H_2O_2 and an increase in morning peak expiratory flow in patients with atopic asthma compared to the placebo group (olive oil) (see Table 2) (Emelyanov et al. 2002).

3.3 Gastrointestinal Protection

The therapeutic efficacy of the CO_2-SFE lipid-rich fraction has also been assessed in gastrointestinal disorders, with significant efficacy in a dextran sodium sulphate-induced inflammatory bowel disease (IBD) model in rats (Tenikoff et al. 2005). The lipid fraction significantly limited body weight loss, reduced disease activity indices and overall morphology of the inflamed intestinal tissue reducing crypt area loss preventing cecum and colon weight loss, all providing potential evidence for successful management of IBD. The lipid fraction was also assessed as a potential treatment in chemotherapy-induced intestinal mucositis in a rat model, using 5-fluorouracil as the toxin; however, the lipid fraction demonstrated only limited efficacy in reducing the symptoms (Torres et al. 2008) (see Table 4).

4 The Role of Intestinal Microbiota in the Therapeutic Activity of *Perna canaliculus* for Inflammatory Conditions

Humans and commensal bacteria coexist in a usually symbiotic relationship with a host to microbe cell ratio of 10:90 %, respectively. It is estimated that the GIT microorganisms collectively make up to 100 trillion cells, tenfold the number of human cells (Lederberg 2000). The collective microbial community is termed the microbiota or the microbiome and it populates specific human environments (e.g. the skin, mouth, nasal cavity, GIT and the urogenital tract). The GIT, skin, urogenital and respiratory systems are extensively colonised by symbiotic microorganisms (Singh et al. 2013). In the human GIT, there is a gradual increase (proximally to distally) in the density and diversity of the microbiota, with the large bowel microbiota representing the most dense, diverse and complex microbial ecosystem known (Tremaroli and Backhed 2012). The genomic content of the GIT microbiome is reported to encode 3.3 million unique bacterial genes, out-numbering the human genome by a factor of approximately 150 (Qin et al. 2010). The human genome, together with its associated microbiome, shares a mutually symbiotic relationship. The microbiota that colonise the GIT regulate normal development and function of the mucosal barriers; assist with maturation of immunological tissues, such as gut-associated lymphoid tissues, promoting immunological tolerance to antigens (foods, environment, pathogens); induce chemical communication to target tissues such as the liver, brain, muscle, adipose tissue, heart and GIT; prevent propagation of pathogenic microorganisms as well as control nutrient uptake and metabolism (Shen et al. 2013).

The GIT microbiota contributes to the metabolism of ingested compounds during the digestive process, including both foods and pharmaceutical drugs, to produce numerous metabolic products. Such metabolites function as signalling molecules between the bacteria and host cells. Metabolites that regulate

host–microbiota dialogue include short-chain fatty acids (SCFA), bile acids (e.g. choline) and lipids (i.e. LPS and peptidoglycan). The genetic richness of the GIT microbiota allows the expression of specific metabolic activities that are not encoded by human DNA (Gill et al. 2006; Egert et al. 2006; Laparra and Sanz 2010), including the hydrolysis and fermentation of dietary polysaccharides (Tremaroli and Backhed 2012). Therefore, the commensal GIT microbiota plays a critical role in human GIT metabolism. The metabolism of *P. canaliculus* by commensal microbial species has not been well explored. However, in vitro analyses have indicated that certain commensal bacteria ferment and metabolise the popular anti-arthritic medication D- glucosamine (Foley et al. 2008; Koser et al. 1961; Wolfe and Nakada 1956; Lutwak-Mann 1941; Faulkner and Quastel 1956). The metabolic capacity of intestinal microbiota can modify bioactive food components altering the hosts' exposure to these components and potentially enhancing or diminishing their health effects. Furthermore, a number of microbiota-based interventions have shown to contribute to human health through maintaining normal microbial composition, improving metabolism and immunity of the gut and by enhancing mucosal integrity and barrier function (Turnbaugh et al. 2006; Gigante et al. 2011). Functional food components such as inulin, are known to influence the growth and metabolic activity of the GIT microbiota and thus its composition and subsequent metabolic capacity (Laparra and Sanz 2010; Campbell et al. 1997; Gibson et al. 2005). The intestinal microbiota is a target of nutritional interventions such as *P. canaliculus*, influencing bacterial viability, growth and metabolic activity (Coulson et al. 2013). Bacterial microbiota may consequently influence biological activity of nutritional supplements. It is proposed, therefore, that the therapeutic activity of *P. canaliculus* is potentially, or in part, due to its interaction with gut bacteria and consequential influence on the host immune system.

The implication of the GIT microbiota in rheumatic diseases has been recognised in in vivo studies. The discovery of a wide variety of bacterial species and bacteria-derived peptidoglycan-polysaccharides (PG-PS) present in synovial fluid from not only reactive arthritis, but also chronic forms of arthritis including RA and OA, indicates that arthritic joints are not sterile as previously thought (Kempsell et al. 2000b). The presence of bacterial antigens within the synovial fluid may play a role in the pathogenesis of several forms of arthritis, other than septic arthritis, such as triggering or exacerbating joint inflammation (Gerard et al. 2001; Kempsell et al. 2000b; van der Heijden et al. 2000; Siala et al. 2009a; Olmez et al. 2001; Carter et al. 2009). Analysis of synovial fluid from OA patients using various polymerase chain reaction (PCR) methods including reverse-transcriptase PCR, broad-range PCR and 16S rRNA PCR, has detected DNA from various bacterial strains. Bacterial DNA that has been detected in OA patients includes *Pseudomonas sp., Shigella sp., Escherichia coli, Chlamydia trachomatis* and *Chlamydia pneumonia* (Olmez et al. 2001; Carter et al. 2009; Gerard et al. 2001). From OA synovial fluid, immunoglobulin G (IgG) antibodies have been detected against *Porphyromonas gingivalis, Prevotella intermedia and Bacteroides forsythus* using enzyme-linked immunosorbent assays (ELISA), while IgA antibodies against *B. forsythus* have also been detected (Moen 2003). Additionally, there is evidence

suggesting that host MHC genes may affect the microbiological milieu of the gut (Vaahtovuo et al. 2003; De Palma et al. 2010). High levels of antibodies directed against antigens of certain gut bacteria in RA patients propose a pathogenic relationship between these bacteria and RA (Scher and Abramson 2011). The exposure to bacterial cell walls may increase the susceptibility to develop arthritis as shown in animal studies (van den Broek et al. 1988; Jonsson et al. 2003). Evidence that bacterial DNA can be detected in OA joints, albeit not as frequently as in RA joints, highlights the importance of patient genetic variability and tolerance. Furthermore, in the case of *Chlamydia trachomatis*, there are several different serotypes that may predict various pathogenic outcomes (Carter et al. 2009). The source of bacteria detected in synovial fluids is not known, but it is suggested they may be derived from environmental sources or from the enteric microbiota (Siala et al. 2009). Periodontal pathogens may also be implicated in arthritic joint inflammation with antibodies to Gram-negative, anaerobic periodontal pathogens such as *Porphyromonas gingivalis, Prevotella intermedia, Prevotella melaninogenica* and *Tannerella forsythia* detected in the serum and synovial fluid of RA patients (Ogrendik 2012). These investigations support the hypothesis that in genetically susceptible subjects, exposure to degraded products of the gut bacteria locally in synovial fluids may cause inflammation. Consequently, it is proposed that bacteria may cause or influence joint disease in a number of ways such as creating persistent infection, inducing autoimmune pathophysiology, producing bacterial antigens or the induction of immune dysfunction (Kempsell et al. 2000a). However, the exact role and the full clinical implications of finding bacterial DNA in arthritic joints are still unknown.

RA patients have altered intestinal microbial profiles that may be relevant to the aetiopathogenesis of RA (Gul'neva and Noskov 2011; Toivanen 2003; Vaahtovuo et al. 2008). RA patients demonstrate decreased *Bifidobacterium*, Lactobacillus and *Bacteroides-Porphyromonas-Prevotella* species, elevated opportunistic *Enterobacteria* and *Staphylococci* species and variable reports of high or low *Clostridium* profiles. Evaluation of gut microbial profile compositions are limited in OA patients, however elevated *Clostridium* and *Staphylococcus* profiles have been found (Coulson et al. 2013). Pain relief medications used by rheumatic patients (i.e. acetaminophen and NSAIDs) may also contribute to altered bacterial profiles in conjunction with their known gastrotoxic effects (Upreti et al. 2010; Cuzzolin et al. 1994; Al-Janabi 2010). In genetically predisposed individuals, environmental factors such as diet, infections and smoking can cause dysbiosis in the GIT microbiota recognised as a microbial imbalance in which one or more bacterial phyla, genus or species overgrow and negatively impact on other beneficial bacteria (Taneja 2014). This dysbiosis may be related to the production of some metabolites as well as the activation of Nuclear Factor-kappa B (NF-kB) pathway that mediates the release of anti-inflammatory cytokines, compromising the integrity of the colonic epithelial cells, increasing gut permeability and consequently affecting health (Chen and Kasper 2014). Therefore, modification of inflammatory conditions such as OA, RA, asthma and IBD may be achieved in part through the refinement of GIT bacterial profiles to reflect a more homeostatic status (Coulson et al. 2012, 2013).

5 Discussion/Conclusion

The therapeutic efficacy of *P. canaliculus* for the treatment of OA, RA and asthma has been a contentious issue with a lack of conclusive evidence-based research. Further scientific investigations are required to evaluate product stability, optimal dosage, novel bioactive compounds and GIT microbiota profiles when assessing the efficacy of *P. canaliculus*. The predominant compound of *P. canaliculus* is protein, which has shown anti-inflammatory and immunomodulating activity in in vitro studies; however, its efficacy has not been investigated in humans yet. Understanding the interaction of the bioactive compounds in *P. canaliculus* with commensal and pathogenic bacterial may facilitate the development of novel interventions to control intestinal and extraintestinal inflammation.

While only two bacterial divisions (*Bacteroidetes* and *Firmicutes*) have been reported to dominate the gut microbiome, thousands of bacterial genera and species inhabit the human gastrointestinal tract. Hence the administration of compounds such as *P. canaliculus* and glucosamine to ameliorate the symptoms of OA and perhaps also RA may involve the actions of the gut microbial cohort to down regulate gut mucosal inflammatory sequalae. Recent clinical data (Coulson et al. 2012, 2013) plausibly suggests that these nutraceuticals may act as prebiotics in the gut, attenuating musculoskeletal inflammatory pain via interactions with the gastrointestinal microbiome.

Advanced sequencing tools/methodologies and experimental approaches have brought novel insights into the mechanisms that promote and maintain gut inflammatory processes that also include auto-inflammatory processes such as in RA. Indeed, it is now possible to locate the site and identity of thousands of bacteria (as well as their functions). This understanding has provided a previously unmatched level of bacterial communities and species detail. For example, animal models studying RA have shown the capacity of specific commensal bacteria to activate pro-inflammatory signalling, which in turn initiate and progress deleterious effects in the joints. The clinical implications of these findings, in parallel with reports that demonstrate humans harbour distinct enterotypes, strongly suggest that musculoskeletal diseases such as RA and the perpetuation of OA may originate in the gut. Certainly this can be plausibly pre-empted for RA from well-characterised studies utilising DNA-parallel sequencing in animal models elucidating possible dysbiosic states (Scher 2010).

If a distinct microbiota profile or pathogen promoted enterotype can be identified, it would then be possible to speculate whether a particular microbiome triggers or drives autoimmunity in genetically predisposed individuals or progresses pro-inflammatory sequale from the gut to the systemic circulation and the musculoskeletal joints. The identification of gut pathogenic commensal profiles could provide insights into the environmental triggers of musculoskeletal diseases and lead to a new understanding of disease pathogenesis, perhaps leading to novel approaches for adjunctive thereby.

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